Inflammatory Modulation in Sepsis – Role of Adenylyl Cyclases

Petter Kirkeby Risøe

Department of Physiology, Institute of Basic Medical Sciences,

and Institute for Surgical Research, Institute of Clinical Medicine,

Faculty of Medicine,

University of Oslo

2012

Series of dissertations submitted to the
Faculty of Medicine, University of Oslo
No. 1482

ISBN 978-82-8264-412-9

Cover: Inger Sandved Anfinsen.
Printed in Norway: AIT Oslo AS.

Produced in co-operation with Akademika publishing.
The thesis is produced by Akademika publishing merely in connection with the thesis defence. Kindly direct all inquiries regarding the thesis to the copyright holder or the unit which grants the doctorate.

Acknowledgements

The present work was begun as a medical student at the Institute for Surgical Research in 2005, and after my graduation in 2010, completed at the Department of Physiology. Throughout these years, I have had to privilege of being surrounded by talented, intelligent and resourceful colleagues, and this thesis would not have come about without their quotidian guidance.

First and foremost, my wholehearted gratitude goes to my supervisor dr. philos Maria Dahle for being my pillar of support as I took my first fumbling steps into the world of medical science. As a tutor, Maria has struck an excellent balance between providing guidance and letting me pursue my own ideas, and she has been a continual source of inspiration to this day. I am also thankful to Professor Guro Valen for taking me under her wings and letting me conduct my research within the intellectually fertile environment that is her group at the Department of Physiology. At the Institute for Surgical Research, Professor Håvard Attramadal has been an invaluable help in sorting out practical matters after I moved to the Department of Physiology.

The financial support from Forskerlinjen has been a necessary prerequisite for this thesis, and for the extension of such stock, I am profoundly grateful. I would also like to thank Assistant Professor Jarle Breivik, former director of Forskerlinjen, for his personal probity and care for the well-being of the students within the program. Norske Kvinners Sanitetsforening has also been a key contributor, without which this work would never have been realized, and during my years as a member of NKS, I have grown increasingly impressed by their diverse and ardent efforts for women's health. In addition, Medinnova, Oslo University Hospital, and Legatet til Henrik Homans Minde have all provided welcome and much-needed funds to the projects contained within my thesis.

The contributions of my coworkers have been immense and stellar by any standards. Dr. philos Jacob E. Wang has bestowed quality advice on strategic scientific choices and academic writing. Yun Yong Wang and Jon Fredrik Stuestøl have wielded their scalpels with such proficiency that their work stand back for no man at Fleet Street (or anywhere else, for that matter). The same goes for Una Ryg, who also afforded me the privilege to complete her early work on adenylyl cyclases in cecal ligation and puncture sepsis, where she had laid down a solid foundation for me to build on. Joanna Ågren has time and time again proved her competency with both *in vivo* and *in vitro* models, and she has been a relished intellectual partner in discussions on everything from methodology to the graphical

representation of statistical results. Ingrid Benedicte Kolseth has time and time again nursed my cell lines to life, providing them with the compassionate care and bonhomous environment needed to make them thrive. Arkady Rutkovskiy put down highly valued work in our attempts at characterizing the changes in adenylyl cyclase proteins, and taught me a thing or two about battle tanks. Most importantly, the above mentioned coworkers have all contributed their good humor and amicable characters, enriching my daily environment with laughter and joy. This also applies to an extended circle of co-workers and office buddies not directly implicated in my projects; none named, none forgotten (but you no doubt know who you are).

I would no doubt have been lost without the first-rate technical assistance of Grethe Dyrhaug, Elin Sletbakk, Signe Flood Kjeldsen and Torun Flatebø. Not only have they lent their brilliant assistance, but they have also proven themselves pedagogic savants by undertaking the challenge of teaching me the laboratory skills required in molecular biology. Early on, I also got substantial technical hints from Professor Henrik Huitfeldt and his group, for which I am very grateful.

A very special thanks goes out to my ravishing wife, who has endured countless evenings and weekends with me away at the laboratory or absorbed by the computer. She has always gone to great lengths to provide me with the time needed to complete the present work, and I am incredibly fortunate to have been blessed with her love and support through this process.

Last, but not least, warm thanks are directed to all healthy volunteers for their sacrificing benefaction. Yanked out of bed at daybreak and abstaining from their dawning cup of Joe, these boys and girls have proven their commitment to the advancement of modern medicine. For their virtual immolation, coffee will always be served to replenish their fluid losses for as long as I remain at the University of Oslo.

Petter Kirkeby Risøe,

Oslo, September 3th 2012

Contents

Abbreviations

A

A2A	Adenosine 2A
A2B	Adenosine 2B
AC	Adenylyl cyclase
AKAP	A-kinase anchoring proteins
ATF	Activating transcription factor

B

BPM	Beats per minute
BCL-3	B-cell lymphoma 3

C

cAMP	Cyclic adenosine 3',5'-monophosphate
CARS	Compensatory anti-inflammatory response syndrome
CBP	CREB-binding protein
CREB	cAMP responsive element binding TF
CHF	Chronic heart failure
CLP	Cecal ligation and puncture
COPD	Chronic obstructive pulmonary disease
COX	Cyclooxygenase
CR	Complement receptor

D

DAMP	Danger-associated molecular pattern
DNA	Deoxyribonucleic acid

E

EBV	Epstein-Barr virus
EPAC	Exchange protein directly activated by cAMP

F

FADD	Fas-associated protein with death domain
FcR	Fragment, crystallizable region

G

GC	Granulocyte
GEF	Guanine nucleotide exchange factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GPCR	G-protein coupled receptor
GTP	Guanine triphosphate

H

HIV	Human immunodeficiency virus
HMGB-1	High mobility group box-1

I

ICAM	Intracellular adhesion molecule
ICU	Intensive care unit
IFN	Interferon
IκB	Inhibitory kappa-light-chain-enhancer of activated B cells
IKK	IκB kinase
iNOS	Inducible nitric oxide synthease
IL	Interleukin
IRAK	IL-1 receptor-associated kinase
IRF	Interferon regulatory factor

J

JAK/STAT	Janus kinase/signal transducer and activator of transcription

K

KC	Kupffer cell

L

LBP	LPS-binding protein
LPS	Lipopolysaccharide
LRR	Leucine-rich repeat
LSEC	Liver sinusoidal endothelial cell
LTA	Lipoteichoic acid
LV	Left ventricle

M

MAMP	Microbial-associated molecular pattern
MARS	Mixed anti-inflammatory response syndrome
MCP	Monocyte chemoattractant protein
MHC	Major histocompatibility complex
MIP	Macrophage inflammatory protein
MMIF	Macrophage migration inhibitory factor
MPO	Myeloperoxidase
MTOC	Micro-tubuli organizing center

N

NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B-cells
NK	Natural killer
NOD-LRR	Nucleotide-oligomerization domain leucine-rich repeat

P

PACAP	Pituitary adenylyl cyclase activating polypeptide
PAF	Platelet activating factor
PDE	Phosphodiesterase
PepG	Peptidoglycan
PKA	Protein-kinase A
PG	Prostaglandin
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cell
PLB	Phospholamban
PRR	Pattern recognition receptor

R

RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
RNP	Ribonucleo protein
RLH	Retinoic-adic-inducible gene I (RIG-I)-like helicases
ROS	Reactive oxygen species
RT-PCR	Reverse-transcriptase polymerase chain reaction

S

SERCA	Sarco/endoplasmic reticulum Ca^{2+}-ATPase
SIRS	Systemic inflammatory response syndrome
SOCS	Suppressor of cytokine signaling

T

TIR	Toll/interleukin-1 receptor
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TRAF	TNF-receptor-associated factors
TGF	Transforming growth factor
TRAM	Trif-related adaptor molecule
TRIF	TIR-domain-containing adapter-inducing interferon-β

U

UTR	Untranslated region

W

WBC	White blood cells

1. List of papers

Paper I

Risøe PK, Wang YY, Stuestøl JF, Aasen AO, Wang JE, Dahle MK. *Lipopolysaccharide attenuates mRNA levels of several adenylyl cyclase isoforms in vivo.* Biochem. Biophys. Acta 2007 Jan;1772(1):32-9.

Paper II

Risøe PK, Ryg U, Wang YY, Rutkovskiy A, Smedsrød B, Valen G, Dahle MK. *Cecal ligation and puncture sepsis is associated with attenuated expression of adenylyl cyclase 9 and increased miR142-3p.* Shock 2011 Oct;36(4):390-5.

Paper III

Risøe PK, Rutkovskiy A, Ågren J, Kolseth IB, Flood Kjeldsen S, Valen G, Vaage J, Dahle MK. *Gender differences in TNFα responses are associated with attenuated adenylyl cyclase expression in male monocytes.* Manuscript submitted for publication.

2. Introduction

2.1 Sepsis and the cytokine theory of disease

Sepsis is a serious medical condition characterized by systemic inflammatory responses triggered by infection, at its most dramatic a process transforming from traceable bacteremia to cataclysmic loss of vital organ function and death within 24 hours. The mortality of severe sepsis with organ dysfunction ranges from 30 to 50%, and according to data from the Centers for Disease Control, it features as the leading cause of non-coronary fatalities in the intensive care unit (ICU) (1). Norwegian estimates operate with an annual incidence of 6000 patients, accounting for approximately 100.000 days of hospital admissions (2).

Sepsis was poorly understood until the early 1970s, when it became clear that manifestations of sepsis could not be solely accounted for through the direct actions of pathogenic toxins. Challenging previously held beliefs assuming the inflammatory response to be uniformly for good, investigations exploring the mechanisms of fever and the acute phase pointed towards proteins produced by macrophages and other immune cells, not pathogens themselves, as a major causative factor underlying tissue damage and organ dysfunction (3). It soon became evident that any understanding of the sepsis phenomena would have to incorporate the host endogenous response as a crucial component of the pathophysiology. These insights led to the eloquent and prescient formulation put forth by a contemporary pioneer, Lewis Thomas:

> "When we sense lipopolysaccharide, we are likely to turn on every defense at our disposal; we will bomb, defoliate, blockade, seal off, and destroy all the tissues in the area… The self-disintegration of the whole animal can be interpreted as a well-intentioned but lethal error. The mechanism is itself quite a good one, *when used with precision and restraint*" (4)

Today, the notion of an exaggerated pro-inflammatory mediator production as a driving force in the pathophysiology of not only sepsis, but other forms of shock as well, has been commonly accepted as the *cytokine theory of disease*. Leaping out of reasonable proportions, the overwhelming production of inflammatory mediators set off a cascade of leukocyte infiltration, endothelial dysfunction, circulatory collapse, hemorrhagic necrosis, multiple organ failure and other physiological calamities (5). However, while our understanding of sepsis has been radically altered over the last three decades, this shift in paradigms has made only modest contributions to its treatment. Current

standard of care remains early goal-directed resuscitation, blood cultures, identifying the source of infection, institution of broad antibiotic regiments and supportive care in the ICU (6). Although considerable gains are possible by way of a more rigid implementation of these principles worldwide, there is an ostensible and dire need for other and more effective approaches in the fight against sepsis (7).

2.2 Innate immunity and sepsis

2.2.1 A brief introduction to innate immunity

The importance of innate immunity for viability in a world dominated by microorganisms is underscored by the early evolutionary origins of the system, which can be traced back to early eukaryotes such as the amoeba. By the time organisms diverged into plants and animals, the main elements of specific pathogen-mediated generic immune responses (e.g. the Toll pathway and NF-κB) had already been hardwired into the genome. This consistency of function, structure, order and purpose over such a wide evolutionary range is one the most impressive examples of conserved ancient evolution (8).

The ingenuity of evolution answered the threat of microbial enemies by developing a defense system based on recognizing unique structural constituents of fundamental importance for the invading microorganisms. Now referred to as pathogen-associated molecular patterns (PAMPs), or sometimes more appropriately microbial-associated molecular patterns (MAMPs) as they are also present in common non-pathogenic organisms, they initiate the innate immune response by binding to pattern-recognition receptors (PRRs) on host cell surfaces (9). Later, the system provided a foundation for the development of additional capacity to detect generic endogenous danger signals (known as danger-associated molecular patterns, or DAMPs) indicating tissue injury, such as heat shock proteins, fibrinogen, fibronectin, hyaluronan, biglycans and high-mobility group box-1 (HMGB1) (10). During pathogen invasion or non-infectious injury such as trauma, PAMPs and DAMPs trigger the activation of different PRRs: 1) Toll-like receptors (TLRs); 2) NOD-LRR; 3) C-type lectins; and 4) cytoplasmic caspase activation and recruiting domain helicases such as RLFs (9). While NOD was originally suggested to act as direct sensors, they are now also thought to function as switches and cellular assembly blocks for such signals of cellular distress, leading to the generation of various multiprotein inflammasomes crucial for inflammatory mediator processing (11;12).

Recognition of cognate activation signals by sentinel immune cells such as tissue macrophages, which are strategically stationed at microbial entry points, result in the production of pro-inflammatory

mediators aimed at attracting more immune cells to the site (8). The recruitment of more activated phagocytes to sites of infection is one of the most important functions of innate immunity. Endothelial permeability and cell-adhesion molecules in vessel walls swiftly increase in order to facilitate this migration towards the foreign invader (discussed more extensively later).

Monocytes are a subset of circulating immune cells that traffic via the blood stream to peripheral tissues, either replenishing stationary tissue macrophages, or when called upon, engage in eradication of pathogens (13). First-responder macrophages that recognize PAMPs or DAMPs call for help by secreting pro-inflammatory cytokines like TNFα, IL-1, IL-12 and IL-23, release chemokines to guide the recruited cells, secrete nitric oxide as part of antimicrobial oxidative processes, and engage in phagocytosis. Depending on the local milieu, macrophages can differentiate into antimicrobial M1 macrophages or wound-healing M2 macrophages; the latter also produce anti-inflammatory cytokines that help resolve and limit inflammation (14).

Granulocytes are the first and most numerous cells to be recruited to infected tissue, and carry storage granules containing proteases that degrade connective tissue in order to allow cells to enter the site of infection, and antimicrobial compounds to effectively neutralize a foreign threat. Neutrophils, the most numerous of the circulating leukocytes, swarm to the site of infection after sensing chemokines, and goes on to release an arsenal of constituents crucial to host defense; a virtual bombardment of reactive oxygen species, antimicrobial peptides, and serine proteases that may destroy not only bacterial and fungal pathogens, but the surrounding tissue as well (15). Mast cells in connective tissue and mucous membranes degranulate within seconds of activation, releasing an array of proteases (thought to be an important part of parasitic defence) and histamine, which initiates vasodilatation and increased vascular permeability to facilitate extravasation of arriving phagocytic cells (16). In addition, mast cells produce eicosanoids, several proinflammatory cytokines and a number of chemokines augmenting the further recruitment of immune cells. Basophils may act synergistically with mast cells in releasing histamine, and eosinophils, recruited by eotaxin chemokines, release free radicals and cytotoxic cationic proteins aimed at fighting pathogens (17;18).

For intracellular pathogens, other strategies are needed. Natural killer (NK) cells terminate other cells that fail to display proper MHC markers at their surface (“missing self”), as this could indicate that they are either broken (e.g. tumor cells) or infected by virus (19). The cytotoxic cells (NK cells, NKT-cells) primarily act by inducing apoptosis or cell lysis in cells that lack proper molecular identification, but also produce an antimicrobial capable of destroying bacteria, eukaryotic cellular pathogens and

parasitic cells by disruption their cell wall (20-23). Dendritic cells and γδ T-cells both serve as bridges to the adaptive immune system (24;25).

The innate immune cells also interact with other defensive mechanisms. For example, the complement system can be cross-activated through inflammatory activation by multiple pathways: either through C3b-binding to the microbe (the alternate pathway), by binding mannose sugars or opsonins on bacterial surfaces (the mannose-binding lectin pathway) or through TLR-C5a crosstalk (26;27). Complement C5a amplifies the production and release of proinflammatory cytokines, enhances phagocytosis in phagocytic cells, stimulates oxidative bursts and granular release in granulocytes, activates the coagulation cascade by inducing tissue factor and acts as a potent vasodilator while increasing the expression of endothelial adhesion molecules (27).

2.2.2 The TLR signaling pathway

Ten mammalian TLRs have been identified since the discovery of the original Toll receptor in the *Drosophilia* fruitfly, and in both families, these PRRs have proved to be of critical importance to the innate immune response (28;29). TLRs are glycoproteins consisting of an extracellular domain containing 16-28 leucine-rich repeat (LRR) modules, a transmembrane domain and an intracellular TIR signaling domain. A large variety of PAMPs induce hetero- or homologous dimerization of the receptors, triggering structural change leading to the recruitment of adaptor proteins to the intracellular Toll/interleukin-1 receptor (TIR) domains (30-32).

TLR4 is the sensor for lipopolysaccharide (LPS), and due to the focus of this thesis, it will serve as an illustration of a TLR signaling process. In conjunction with its coreceptor MD2, TLR4 is specific for lipid A, the main inducer of biological responses to LPS (33-35). The LPS molecule bind exclusively to MD2, and TLR4 and MD2 interaction is mediated mainly by ionic and hydrogen bonds in two oppositely charged patches (36;37). Ligand binding induces the dimerization of the complex, and subsequently initiates signaling. In addition, two accessory proteins are needed to recognize LPS at physiological concentrations (38-40). Plasma LPS-binding protein (LBP) extracts LPS from the outer membrane of Gram-negative bacteria or from vesicles continually shed during bacterial growth, and transfers it to CD14 in a monomeric form (41). Upon binding, two CD14s, which can exist in a soluble form or as a GPI-anchored protein on the cell membrane, form a dimer containing 22 LRR modules which in shape and size appear to be comparable to the TLRs (42;43). Because CD14 has no intracellular signaling domain, LPS is transferred to the TLR4-MD2 complex to initiate immune responses (36;41). Dimerization of the extracellular domains of the activated TLR4-MD2 complex is then thought to

induce dimerization of the intracellular TIR domains, leading to proper orientation or conformational change allowing for downstream signaling mediation (36).

The signaling cascade initiated by activation of the TIR dimers elicits major transformations of cellular transcription. Broadly, two key regulators of inflammation – Nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) and Interferon regulatory factor (IRF) 3 – are activated by the recruitment of two sets of adaptor proteins initiating different signaling pathways; MyD88/Mal and TRIF/TRAM, respectively (44-46). IRF3 activation typically induces interferons (IFNs) implicated in anti-viral defense, and will not be further discussed in this thesis. MyD88 protein and IL-1R associated kinase pathways activate NF-κB, a transcription factor constituted by homo- and heterodimers of the Rel protein family. Latent NF-κB molecules are sequestered in the cytoplasm by IκB proteins. TLR-activation induces degradation of the IκBs in proteasomes, unmasking the nuclear localization signal of NF-κB and resulting in nuclear translocation, binding to NF-κB motifs and the transcription of more than 150 genes. NF-κB is considered a key switch for changing the intracellular physiology from its normal steady-state to inflammatory activation (9).

The engagement of TLR4 by LPS and the subsequent signal transduction has been demonstrated to turn on more than a thousand genes, of which more than 75 % are MyD88-independent (47). Within the scope of a few hours, the gene expression profile of the activated innate immune cell is radically transformed following the recognition of a PAMP or DAMP signal. The first category (class I) of transcription factors induced by PRR binding occurs within 0.5-2 hours, and controls the induction of the primary response genes, including NF-κB, IFN-regulatory factors and cAMP-responsive-element-binding protein 1 (48). These initial transcription factors are activated by posttranslational modification and nuclear translocation after the PRR signal. The second round of transcription occurs from 2 to 8 hours past the initial receptor binding, and depends on (class II) transcription factors induced during the primary response, as well as secondary response genes. Among the proteins resulting from this second round of activation are some of the most well-known pro-inflammatory mediators; tumor necrosis factor α (TNFα), interleukin (IL)-1, IL-6, and IL-12, monocyte chemoacttractant protein (MCP), macrophage inflammatory protein (MIP)-1α and -1β, and inducible nitric oxide synthease (iNOS) and cyclooxygenase (COX)-2 (49-53). In the secondary response, which take place post 8 hours after first contact, the LPS-inducible negative feedback loops are activated and the molecular picture changes towards a state of anti-inflammation, with release of anti-inflammatory cytokines such as transforming growth factor (TGF)β and IL-10, nuclear hormone receptors such as liver X receptors (LXRs), peroxisome proliferator-activated receptors (PPARs), vitamin D receptors, and cAMP (48). The nuclear receptors integrate the control of inflammation

with various physiological functions, such as metabolism, and inhibit inflammation through multiple mechanisms. Inducible negative regulators working at the transcription level of primary response genes are also upregulated; most importantly, IκBNS (controls IL-6, IL-12, and IL-18) and B cell lymphoma 3 (BCL-3, which controls TNF, IL-10 and IL-1β) (48).

TLR expression is significantly up-regulated in patients with sepsis, as well as in states abundant in DAMPs (e.g. trauma, thermal injury) (54-57). It has been proposed that TLR augmentation primes the innate immune system for enhanced TLR reactivity, predisposing to an observed increase in LPS-mediated mortality after DAMP-intensive events (58). The existence of positive feedback loops between DAMPs and PAMPs may represent the molecular basis for the phenomenon that infections, traumas and nonspecific stress factors sometimes triggers flares of systemic inflammation, accompanied by baleful consequences if they spiral out of control (9;55;59).

2.2.3 SIRS, MARS and CARS

When fighting off foreign invaders or reacting to signals of endogenous tissue damage, the innate immune system operates as a cascading process that includes the activation of cells with the potential to cause considerable collateral damage to surrounding tissues as they indiscriminately barrage their environment with reactive oxygen species, antimicrobial peptides, and serine proteases. If the site of infection is extensive enough, dead bacteria and host cells both release vast amounts of PAMPs and DAMPs in the blood stream, which together with the increasing levels of pro-inflammatory cytokines produced locally serve to activate systemic inflammatory responses, such as fever, acute phase protein release, and reinforcement of the pro-inflammatory signal through the outpouring of cytokines from sources beyond the local tissue, such as endothelial cells, circulating monocytes or even remote tissue macrophage populations such as the Kupffer cells of the liver (60).

Clinically, the spill-over from local to systemic, which in severe cases can be viewed as loss of control over the pro-inflammatory cascade, can result in a condition referred to as the systemic inflammatory response syndrome (SIRS): heart rate > 90 bpm; body temperature < 36 or > 38 °C; tachypnea > 20 breaths per minute or P_aCO_2 < 4,3 kPa; WBC count < 4 x 10^3 or > 12 x 10^3 cells/mm^3 or > 10 % immature neutrophils). This clinical definition is generally taken to reflect systemic inflammation where no definite source of infection has been identified (61). Although it is generally agreed that its positive predictive value is unsatisfactory and more elaborate diagnostic consensus criteria for sepsis have been developed, for the purposes of this thesis is it sufficient to use the putative rule of thumb that sepsis is SIRS with an infectious cause (62).

In the course of our present decade, the *cytokine theory of disease* and its concept of SIRS have been supplemented, albeit not directly challenged, by the concepts of mixed anti-inflammatory response syndrome (MARS) and compensatory inflammatory response syndrome (CARS). Incorporating the fact that most patients receiving current standard of care do not die from the initial septic insult, but more often from a "second" or "third hit" caused by secondary infection acquired during their stay in the ICU, this model presumes that the overproduction of pro-inflammatory mediators evolves into a state with high signaling activity on the part of both pro- and anti-inflammatory cytokines, MARS. Subsequently, MARS gradually gives way to a more prolonged homeostasis characterized by exaggerated anti-inflammatory release causing severe immunodepression, CARS, predisposing for secondary infections and renewed bouts of disease activity in the already torpid patient (63;64).

This reappraisal of Lewis Thomas theory of uncontrolled hyperinflammation as the sole contributor to septic shock pathophysiology is based on a substantial evidence showing that most septic patients went to a situation of dysregulated and debilitated innate immune responses. Often referred to as immunoparalysis, the condition was found to last from three to seven days in monocytes after trauma [163]. During this period, activated CD4 helper T cells undergo a shift in their secretion of cytokines, and the initial release of pro-inflammatory cytokines such as TNFα, IFNγ and IL-2 produced while the cell is in type 1 (Th1) mode gives way to the antagonistic type 2 (Th2) mode, where production of anti-inflammatory IL-4 and -10 dominates (64). Both in human and animal models, CARS has been characterized by a cytokine profile of predominantly anti-inflammatory nature, monocyte deactivation, dysfunction of dendritic cells, impairment of neutrophils, and lymphocytic anergy and apoptosis (65;66).

However, the orderly model of MARS and CARS is poorly supported by clinical or *in vivo* evidence (67). An experimental animal study examined the inflammatory alterations preceding lethality in late sepsis, failing to find evidence supporting a linear connection between SIRS, MARS and CARS (68). This publication strengthened the claim that mechanisms leading to death in the early, pro-inflammatory shock differ from death during the later, hypoinflammatory stage (69). Instead, the SIRS-to-CARS transition may rather be a constantly fluctuating oscillation between hyper- and hyporesponsiveness in different patients (65).

2.2.4 Gender aspects of sepsis

Numerous articles point to a gender dimorphism in the susceptibility for and morbidity from sepsis, although the precise role of gender has been subject to considerable controversy and conflicting reports (70-81). Sepsis, itself a catch-all term for what may represent more than one immunological

disequilibrium, are associated not only with a wide range of confounding factors, such as lifestyle activities, risk behaviors, ethnicity, local microbial flora, but also healthcare access and delivery. Although most English-language articles in the field report gender as a demographic variable, only a minority examine the effects of gender on health outcomes (82). Some authors have suggested that gender differences in symptoms, presentations of illness, or diagnostic bias make women are less likely to receive advanced intensive care interventions, and a number of the studies do demonstrate the existence of a gender disparity in treatment (72;76;78).

The data on effects of sex hormones on the various components of human immunity *in vivo* is conflicting, suggesting a spectrum of cytokine production in females from both more and less pro-inflammatory action compared to males (81;83-86). However, most studies have not controlled or standardized subjects with respect to menstrual phase and hormonal contraceptives, which have both been shown to have profound effects on female immune physiology (87;88).

Among the conflicting reports on gender dimorphism on septic mortality there are two broader observations that are notable. The first is that the incidence of sepsis is considerably higher in males in a majority of the unmatched studies, suggesting that men are more likely to progress from bacteremia to sepsis relative to women (70-72;74;76;79-81;89). The second observation is that those studies examining premenopausal women separately show that they have consistently higher survival rates compared to men of comparable age (70;72;74;76;79;81). However, the survival advantage conferred by female gender appears diminished or disappeared altogether in the sixth decade of life, and some studies even indicate worse outcomes for older women (71;78;80;89). As these changes appear to correspond in time with the menopause it seems plausible that they may be related to the immunological effect of female sex hormones.

2.3 The cyclic AMP second messenger system

2.3.1 A brief introduction to the cAMP signaling system

In the 1950's Southerland and Rall detected that intracellular levels of cyclic adenosine monophosphate (cAMP) were elevated when adding hormones, and serendipitously made the first discovery of intracellular signaling. They demonstrated that cAMP is synthesized from adenosine triphosphate (ATP) by membrane-bound adenylyl cyclases (ACs), activated by transmembrane receptors via G-proteins. Sutherland was awarded the 1971 Nobel Prize for his work, which would prove to be the first of five Nobel Prizes recognizing research on this molecule (90). Half a century

later, the cAMP signaling pathway is referenced as the archetypical second messenger system, regulating a wide range of functions in all mammalian cells in a precise and specific manner.

With one exception, the AC isoforms are all membrane-bound and regulated by extracellular signals through transmembrane G-protein coupled receptors (GPCRs) and G-proteins. Hundreds of different GPCRs exist, but it has revealed that the interactions between certain GPCRs and ACs at the cellular level is highly specific (91). Simplifying greatly, G-proteins (each made up of an α, β and γ subunit) released by an activated receptor are classified according to whether they stimulate (Gs) or inhibit (Gi) cAMP production (92). Some ligands best known for their activation of Gs-coupled GPCR ligands include epinephrine, norepinephrine, nicotine, histamine, serotonin and certain COX-derived prostaglandins (particularly PGE_2 and $-I_2$, the latter also known as prostacyclin), while examples of Gi-coupled GPCR ligands are chemokines CCR1-1 and CXCR1-6, as well as leukotrienes B4, C4 and D4 (93). Recently, it has been revealed that GPCRs may continue their signaling via the cAMP system after being internalized (94).

The ten isoforms of AC are countered by more than 40 different phosphodiesterase (PDE) isozymes (95), which through distinct expression and regulatory profiles together tune the levels of cAMP in the cell. PDEs and cAMP target proteins can associate with the AC-G-protein membrane complexes, forming defined subcellular compartments where a localized pool of cAMP exerts its effects (91;95). The diversity of cAMP effector molecules, as well as lipid rafts, scaffolding, adaptor and anchoring proteins, contributes to the complexity of the pathway (96-98). A number of methods have now been developed to study such microdomains at the single-cell level (97;99). Simplified illustrations demonstrating different cAMP signaling routes are shown in Figure 1.

There is ample scientific evidence for highly divergent, if not completely opposite, functions of the different downstream elements to cAMP; the protein kinase PKA, the guanine nuclear exchange factor Exchange protein activated by cAMP (EPAC) and cyclic nucleotide-gated ion channels. Ion channels dependent on cAMP has been found in diverse tissues for Na^+, K^+, Ca^{2+}, Cl^- and HCO3 channel function (100-102). Cyclic nucleotide gated ion channels are regulated directly by the binding of cAMP to their cytoplasmic tail, and A-kinase anchoring proteins (AKAPs) seem to be essential in creating the necessary microenvironments for this to happen (103-105). Also, specific cAMP analogues have been developed to activate PKA and EPAC separately, which has given rise to studies allowing for their separate study (102;106).

Together, these discussed features give the cAMP signaling system flexibility and spatiotemporal precision to act upon multiple intracellular processes. In different cell populations, cAMP has been

demonstrated to serve crucial signaling functions in processes as diverse as cell growth control, survival, motility, gene expression, cell-cell communication, adaptive immunity, cardiac and smooth muscle contraction, metabolism, secretory processes and memory (90;107;108). Given these pleiotropic actions of cAMP it is not surprising that pharmacological manipulation of the cAMP pathway has proven benefit in a wide range of human disease, exemplified in conditions ranging from asthma and depression to HIV infection and erectile dysfunction (109-111).

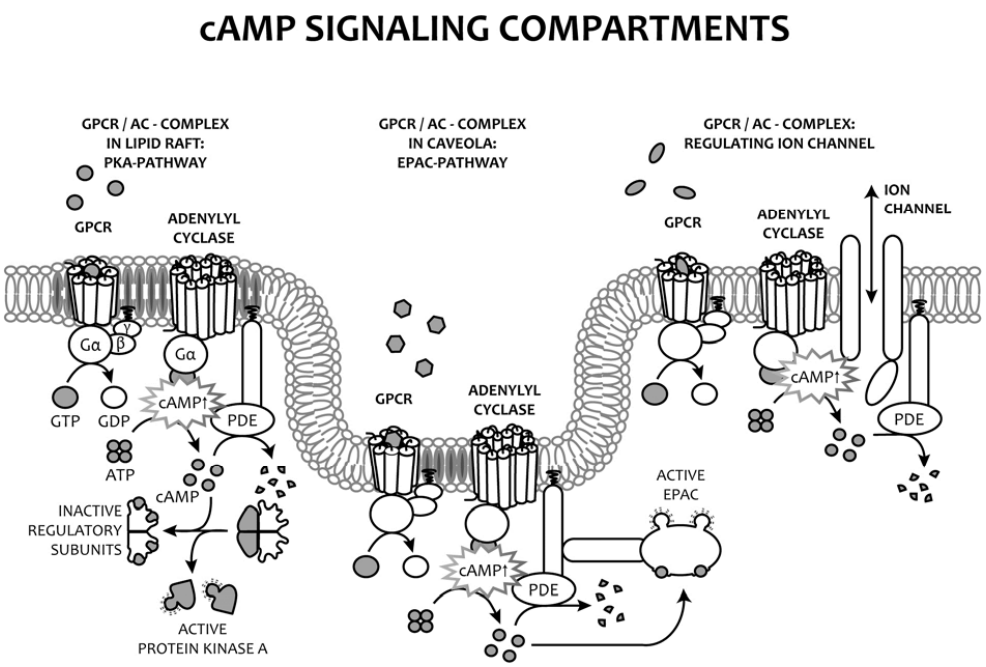

Figure 1: cAMP signaling compartments. *GPCRs, ACs, PDEs and cAMP target proteins, such as PKA, EPAC or cAMP-regulated ion channels, associate and form defined subcellular signaling compartments. Please note that the configuration shown in the diagram serves just to exemplify signaling compartments and their potential localizations in the membrane, and that it is not intended to indicate a link between membrane localizations of the compartments and down-stream mediators.*

2.3.2 Downstream effectors of cAMP

PKA, the first mediator to be identified, is an ubiquitously expressed heterotetramer consisting of two catalytic and two regulatory subunits (112). Two isoforms, type I and II, differ in their regulatory subunits, RI and RII respectively. In addition, four catalytic subunits have been identified by molecular cloning. All are differentially expressed, forming homo- and heterodimers, and they are likely to contribute to increased signaling specificity (113). On binding to two cAMP molecules by each regulatory subunit, the inactive tetramer dissociates into one dimer of R subunits and two active catalytic subunits that phosphorylate various target proteins (114). A-kinase anchoring proteins (AKAPs) have been shown to harbor PKA isoforms in particular subcellular compartments, associating with PDE, and upon GPCR-activation, stabilizing defined microdomains in lipid rafts or caveolae at cellular membranes (115-117). Among the main functions of PKA is the phosphorylation of the transcriptional activation domain of the cAMP-responsive-element binding protein (CREB) family of transcription factors, which then acts with cofactors such as CREB-binding protein (CBP) or p300 to initiate transcription of the CREB regulon, a collection of target genes involved in cellular processes such as proliferation, survival, differentiation and inflammatory responses (118).

In 1998, a vital step towards a fuller understanding of the cAMP signaling system was taken when EPAC was discovered as a PKA-independent signal mediator (119). Two EPACs are now known, and both are specific guanine nucleotide exchange factors (GEFs) for the Ras GTPase homologues, Rap1 and Rap2 (119;120). Like most other other small GTPases, they act as molecular switches, cycling between a GDP-bound inactive and a GTP-bound active state regulated by the balance between activating GEFs and inactivating GTPase-activating proteins (GAPs). EPAC2 is predominantly expressed in the brain and adrenal gland, while EPAC1 is present in kidney, ovary, thyroid and leukocytes (92). By using the EPAC-selective cAMP analogue 8-pCTP-2-O-Me-cAMP it has been shown how a great many effects ascribed to PKA in previous publications are actually mediated by the EPAC pathways; E- and VE-cadherin-mediated cell-to-cell adhesion, integrin-mediated adhesion, monocyte chemotaxis, Ca^{2+}-induced exocytosis and FcR-mediated phagocytosis (106;114;121-126). However, despite the increasing body of evidence implicating EPAC in many of the important anti-inflammatory effects of cAMP, the exact molecular basis for these processes has yet to be elucidated.

2.3.3 Flexibility and spatiotemporal precision in the cAMP system

The fact that cAMP signals could elicit differential downstream responses was first observed more than three decades ago, and it has since become clear that the system can achieve a remarkable specificity and flexibility through spatial control of the components involved in signal transduction

(96). Up until the current decade, studies into the intracellular workings of cAMP signaling was hampered by a lack of adequate tools; however, in recent years there has been a burgeoning of techniques and biosensors allowing for investigation of localized cAMP gradients within cells (99). These have revealed an intrinsic system where macromolecular complexes such as mAKAP can serve as nodes capable of integrating multiple cAMP signals with those of calcium and MAP kinases through the creation of subcellular cAMP pools with sequestered PKA and EPAC subpopulations which act as regulators of spatially localized signaling (103;127). Different R subunits construe PKA holoenzymes with distinct physiological and biological properties, which can then sense spatially discrete pools and selectively phosphorylate downstream effectors (96). Many of the processes regulated by EPAC are modulated by PKA, and there are indications of considerable cross-talk between the two main effector pathways (128;129). PDEs provide the sole means of cAMP degradation in cells, and with more than 40 identified isoforms, they are believed to provide underpinning for the compartmentalization (127). While performing the same basic function of hydrolyzing cAMP, the individual isoforms are characterized by targeting signals which allow precise localization and an intense variety in regulatory properties (130).

Investigations of spatiotemporal aspects of the second messenger system within molecular cardiology have demonstrated how these principles can coalesce into the working fundament of a precise intracellular signaling system consisting of building blocks ubiquitously available across mammalian cell types (96;131). However, the effects of cAMP signal compartmentalization in innate immunity is for the most part still unexplored.

2.4 The role of cAMP in inflammation

Manipulation of intracellular cAMP levels has been shown to suppress innate immune functions of monocytes, macrophages and neutrophils through the modulation of three key effector functions; generation of inflammatory mediators, phagocytosis and intracellular killing of ingested pathogens (90). In addition, there is strong evidence implicating cAMP in the regulation of transendothelial migration (114).

2.4.1 Modulation of mediator generation

There is a potent inhibitory effect of cAMP signaling on the endotoxin-mediated production of the pro-inflammatory mediators TNFα and leukotriene B4, the leukocyte attracting and activating chemokines IL-8, MIP-1α and -1β, and the activator of cytotoxic responses in T and NK cells IL-12 (p40), in monocytes and macrophages (132-137). This inhibition is considered to be primarily

responsible for the anti-inflammatory effect of cAMP-elevating drugs in vivo. TNFα acts as a crucial early initiator of immune responses to pathogens, and is inhibited at a transcriptional level by a mechanism which is not completely understood, but has been proposed to involve the cyclic AMP response element (CRE)-binding inducible cAMP early repressor (138). Competitive inhibition of NF-κB by activated CREB for limiting amounts of CBP/p300, which is needed to acetylate the RelA subunit of NF-κB, has been proposed as another pathway for attenuated production of proinflammatory mediators (139;140). Downstream in the cAMP signaling pathway, constitutive cAMP elevation by various forms of PDE inhibition efficiently impair TNFα expression and release. The isoform PDE4B was demonstrated to play a central role when macrophages from PDE4B-deficient mice were shown to elicit impaired LPS-stimulated TNFα production (141). In line with this, reactive oxygen species (ROS)-mediated TNFα response was recently reported to be more or less obliterated by PDE4 inhibitors (142).

In contrast, cAMP has been shown to potentiate the anti-inflammatory IL-10 response to LPS challenge in macrophages, boosting the release of an important factor for protection against the onset of septic shock (132;143). CREB, together with another transcription factor AP1, seems to play a role in binding to the IL-10 promoter when phosphorylated (144). Global cAMP elevation also enhances the expression of the suppressor of cytokine signaling (SOCS) 3 gene, a brake on inflammatory IL-6 production induced by IL-6 and IL-10 together, in peripheral blood mononuclear cells (PBMCs) and neutrophils (135;145).

The cAMP-mediated regulation of inflammatory mediator production in myeloid cells appears to be effected by both PKA- and EPAC1-dependent pathways. Downstream to PKA, the transcriptional regulation has been shown to involve the CRE-binding protein and CAAT/enhancer binding protein (C/EBP) transcription factors attaching to genetic promoter elements (146;147). Multiple pathways for the immunomodulatory effects of EPAC has been suggested, from its influence on extracellular matrix proteins such as laminin, fibronectin and hyaluronan that are feeding back the signal as tissue injury signals, to the involvement of ERK and C/EBP in bringing fourth the observed induction of SOCS3 (107;148).

2.4.2 Impairment of phagocytosis

Phagocytosis involves a highly regulated sequence of signal transduction events that lead to cytoskeletal and membrane rearrangement and eventually particle engulfment (90). Raising global cAMP levels in myeloid cells have been demonstrated to suppress the opsonin-dependent Complement receptor (CR) and Fragment crystallizable region receptor (FcR), as well as opsonin-

independent scavenger receptor-mediated phagocytosis (149-151). However, the mechanisms of cAMP-mediated inhibition in phagocytic function have not been completely defined. cAMP has been shown both to inhibit and promote F-actin polymerization during FcR-driven phagocytosis (152;153). Other reports points toward regulation on the level of receptor expression (149;154).

EPAC1 has also been demonstrated to mediate the cAMP-dependent reduction of FcR-phagocytosis in alveolar macrophages (155). However, reports indicate that both PKA and EPAC1 may counter-regulate phagocytosis in different cell types (151). Par example, PKA seems to regulate FcR phagocytosis in circulating monocytes, while both EPAC1 and PKA inhibit this function in monocyte-derived classically activated macrophages (133).

In addition, cAMP also appears to regulate phagolysosome maturation. Increased cAMP levels decreased phagosomal formation and acidification in a PKA-dependant manner (156;157). Interestingly, several components of the cAMP signaling pathway were found to be localized to the phagosome. PDE4 was found to be colocalized with PKA in the forming phagosome in neutrophils, and both EPAC1 and Rap1, its downstream effector, associated with phagosomes containing IgG-opsonized targets in alveolar macrophages (158;159).

2.4.3 Modulation of microbicidal activity

Granulocytes contain stores of antimicrobial proteins, ROS and proteases for the purpose of tissue extravasation and bacterial killing. In order to strictly limit the release of granule content, degranulation is induced by adherence in combination with locally produced stimulators including TNFα and granulocyte-macrophage colony-stimulating factor (GM-CSF).

cAMP elevation suppress the microbicidal capacity of leukocytes toward bacteria, viruses, fungi and eukaryotic parasites by mechanisms which are vaguely understood (133;155;160-164). In vivo, the cAMP-mediated inhibition of TNF-production will indirectly attenuate degranulation, but cAMP is also known to inhibit exocytosis of adherent neutrophils directly. In neutrophils from knock-out mice obtained by bronchoalveolar lavage, PDE4B and -4D, but not -4A, was demonstrated to regulate several aspects of their function (165).

Bactericidal ROS release is down-regulated by a myriad of cAMP-elevating agonists. This effect is associated with, and has been proposed to involve, two pivotal steps in NADPH oxidase activation, namely the phosphorylation and translocation of the cytosolic p47phox subunit (161;166;167). Both PKA and EPAC-1 activation have been implicated in the inhibition of ROS production in alveolar macrophages (155).

In the regulation of reactive nitrogen intermediate generation, cAMP levels have been found to have a pivotal, if somewhat poorly understood and possibly dual, role in regulation of iNOS expression and NO production (168-171).

2.4.4 cAMP signaling in leukocyte transendothelial migration

Leukocyte extravasation is tightly regulated by bidirectional signaling in both leukocytes and vascular epithelium. The mechanisms controlling this migration is a combination of chemokine-mediated attraction, integrin-dependent surface communication between leukocytes and endothelial cells, (rolling and adhesion), and migration across the endothelium. Regulation of the macrophage production of several leukocyte-attracting chemokines (IL-8, MIP-1α, MIP-1β) by cAMP plays an indirect role, but cAMP signaling in the recruited leukocytes also controls this process. cAMP signaling appears to regulate leukocyte infiltration in two opposite directions, depending on whether the downstream cAMP signaling mediator activated is PKA or EPAC (125).

Integrin-dependent cell adhesion to fibronectin have been demonstrated to be induced by cAMP via Epac-mediated activation of Rap1, and it was recently reported that loss of Epac1 in monocytes reduced activation of the αMβ2 integrin, and that Epac1 promoted monocyte adhesion to vascular endothelium (124;172). However, PKA-mediated mechanisms have been reported to inhibit extravasation of leukocytes. In neutrophils and eosinophils, induction of cAMP by the PDE4 inhibitor rolipram blocks the surface expression of αMβ2-integrin and inhibits shedding of the L-selectin CD62L, which is a prerequisite for efficient rolling (173). These effects were PKA-dependent. Integrin-dependent adhesion was inhibited by PKA, an effect lost when subcellular PKA anchoring was disrupted (174). Likewise, in monocytes, cAMP inhibits surface expression of β2-integrins and CD62L shedding via PKA (133). As transendothelial migration requires the disassembly of the tight junctions that retain endothelial barrier function, the vital role of cAMP-signaling in maintaining vessel wall integrity should be noted (discussed more extensively later).

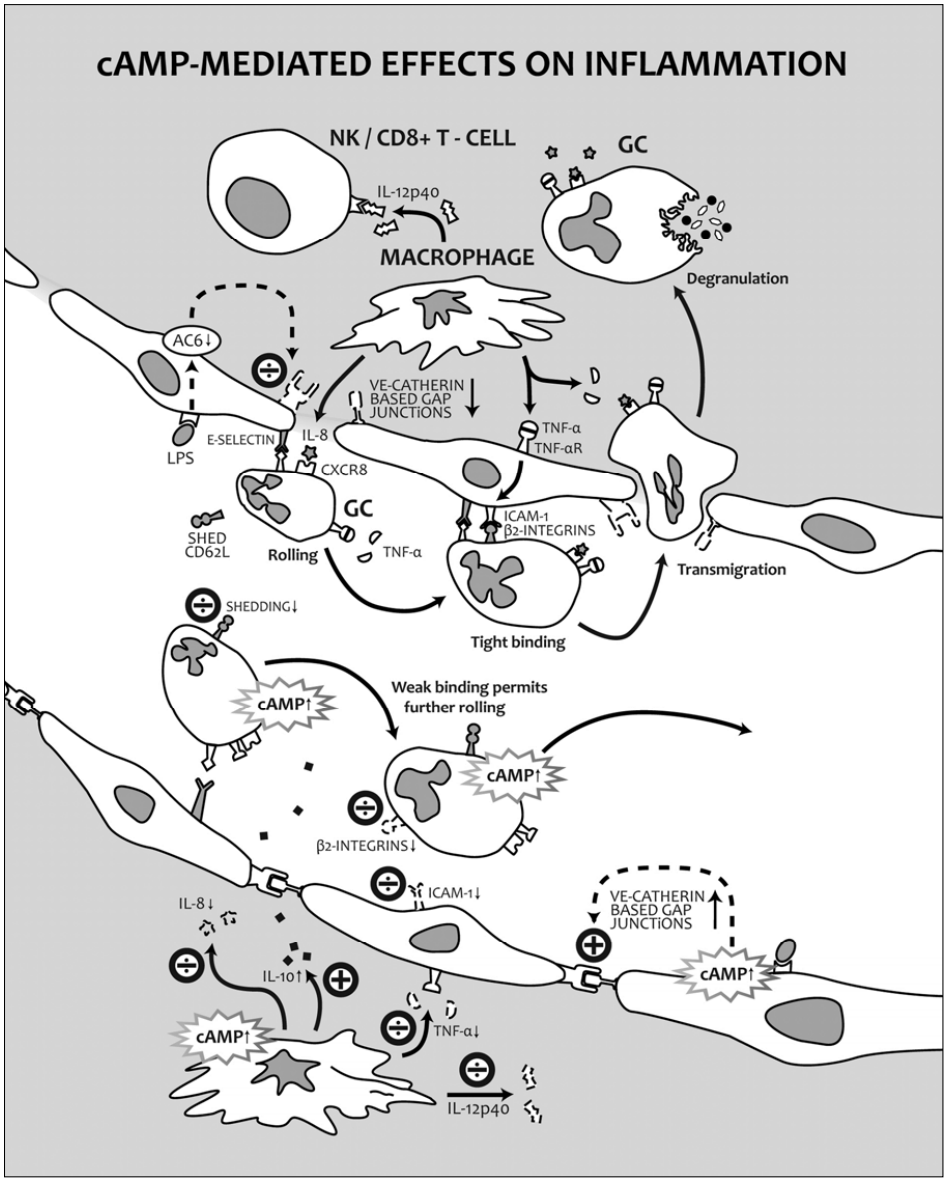

Figure 2: cAMP-mediated effects on inflammation. *The cAMP signaling system plays several important roles in the innate immune response. The upper half of the depicted vessel exhibits a normal inflammatory response; endotoxin dissolves epithelial gap junctions, tissue macrophages call for circulating immune cells, and endothelial cells display adherence molecules that facilitate the extravasation of activated neutrophils that degranulate out in the tissues. The lower half depict the same situation, but with a generalized rise in cAMP levels; pro-inflammatory cytokine release is reduced, endothelial gap junctions stay intact, and fewer adherence molecules are expressed, leading less leukocyte rolling and extravasation.*

2.5 Dysregulation of cAMP in sepsis and other inflammatory states

2.5.1 Altered cAMP levels in sepsis and endotoxemia

The first hints of cAMP dysregulation in sepsis came as serendipitous findings from studies of the hepatic glycogen metabolism in the 1970s, where altered cellular levels of cAMP were noted in rat endotoxemic models (175;176). Some years later, trials of prostaglandin synthesis inhibitors confirmed abnormal liver values of cAMP in dogs, as well as decreased AC responses to various GPCR ligands (177;178). Disturbances in myocardial cAMP levels during endotoxemia were also suggested to be of importance in the autonomous response to inflammation, a concept which has been revived in this decade (3;179).

In the late 1990s, comparisons between PBMCs from healthy controls, critically ill non-septic patients and septic patients revealed a 40 % attenuation of β-adrenergic receptors (βARs) in the two latter groups, as well as reduced basal levels of cAMP. However, when cAMP production capacity was tested using the AC activating agents NaF or forskolin, there was a significantly lower responsiveness in the septic patient group attributed to a heterologous desensitization of AC activity (180). This finding was confirmed by exposing PBMCs drawn from healthy human subjects to serum from patients with severe sepsis or septic shock, which conferred reduced cAMP production potential (most markedly in the shock serum group) compared to serum from healthy controls (181).

In knock-out models of mice, the inactivation of different cell mechanisms for raising intracellular cAMP levels suggest that they may be closely linked to the development of SIRS and sepsis. Deficiency in the cAMP-inducing adenosine 2A (A2A) or pituitary adenylyl cyclase activating peptide (PACAP) receptors has been shown to make mice highly susceptible to developing sepsis (182;183). PDE4B knock-outs, but not knock-outs of the highly related PDE4A or PDE4D isoforms, exhibit a marked improvement in survival after receiving a high dose intraperitonial injection of LPS, as well as a > 50 % reduction in macrophage TNFα production (141).

2.5.2 Using cAMP pathway manipulation to evade host defences

Underscoring the importance of cAMP as a key regulator of innate immune function is the finding that several pathogenic microorganisms have evolved mechanisms to exploit host cell cAMP signaling as a virulence factor. By employing toxins that disrupt the normal regulation of cAMP production directly or indirectly, bacteria can disable host cell phagocytosis, intracellular killing and inflammatory mediator generation, all of which give them an advantageous edge in establishing infection (90;184).

Probably the best known microbial pathogen that uses cAMP amplification as a virulence factor is *Vibrio cholerae*. The cholera toxin is a heterodimer, and binding by one subunit to the cell surface results in translocation of the other subunit into the cell. When the toxin has reached the cytoplasm, it catalyzes the ADP-ribosylation of the α subunit of the stimulator G protein and facilitates constitutive activation of AC enzymes. The resulting cAMP accumulation in the gut epithelial cells is recognized as the chief cause of massive diarrhea (185). Similarly, the heat labile toxin of *Escherichia coli* exert it unwholesome effects in a resembling manner (184). Both toxins have been shown to inhibit phagocytosis, intracellular bacterial killing, pro-inflammatory mediator production and chemotaxis in myeloid cells (160;186-188).

Bordetella pertussis is a small, gram-negative bacillus that causes the highly contagious childhood disease whooping cough, and it provides an illustrative example of how overwhelming the cAMP system can derail innate immune defences (184;189). Two toxins secreted by *B. pertussis*, the PT and CyaA toxin, both act to raise host cell cAMP, albeit by separate mechanisms (189;190). The PT toxin catalyze ADP ribosylation of the α subunit of the inhibitory G-protein, while the CyaA toxin enters the cell, is activated by eukaryotic cofactors such as calmodulin and then act as an exogenous AC enzyme, mediating unregulated conversion of cellular ATP to cAMP. These toxins have been demonstrated to impair phagocytosis, reduce ROS generation, suppress chemotaxis and attenuate cytokine production in neutrophils and macrophages (63;191-194). Three other calmodulin-dependent bacterial AC toxins have been identified; the edema factor of *Bacillis anthracis*, the ExoY of *Pseudomonas aeruginosa* and the AC toxin of *Yersinia pestis*. In addition, *Bacillis subtilis* secretes acrylpeptides that inhibit phosphodiesterases to order to raise cAMP levels (184). Taken together, these findings illustrate the diversity of cAMP manipulation techniques evolved by pathogenic microbes to incapacitate our innate first line of defense.

2.6 MicroRNA and the innate immune system

2.6.1 MicroRNAs as key regulators of cellular fate and function

MicroRNAs (miRNAs) are an abundant gene family of small, noncoding ribonucleic acids that over the last decade have radically augmented our understanding of the molecular mechanisms that regulate gene expression. Present in virtually all species, miRNA-mediated post-transcriptional repression of protein-coding genes represents a fundamental layer of genetic programming, able to exert control over diverse and complex cellular functions such as development, proliferation, differentiation, cell fate determination, apoptosis, signal transduction and the maintenance of homeostasis (195). Although not recognized as a distinct class of biological regulators until nearly a decade after first

being discovered by Ambros, Lee and Feinbaum in 1993 (196), their high conservation across eukaryotic organisms point towards ancient origins and a crucial role in genetic regulation. To date, about 700 different human miRNAs have been discovered and they are estimated to target around 60 % of mammalian genes (197). Many of the miRNAs exhibit a distinct cell- or organ-specific expression, underscoring their importance as key players in the establishment and maintenance of cell fates, and abnormalities in their expression have been observed in a wide array of human diseases (198).

2.6.2 Biogenesis and mechanism of action

Mature miRNAs are small, single-stranded RNA molecules of approximately 22 nucleotides that regulate mRNA stability and translation through antisense RNA-RNA interaction (199). In brief, miRNA genes are typically transcribed by RNA polymerase II into long primary transcripts (pri-miRNAs) that form a stem-loop structure and which can be hundreds or more than a thousand nucleotides long (195). In the most common ("canonical") pathway, a microprocessor complex with a catalytic core composed of the Drosha and DiGeorge syndrome Critical Region 8 (DGCR8) proteins crop and polyadenylate the primary transcripts in the nucleus. Resulting from these processes are ~ 70 nucleotide precursor-miRNAs (pre-miRNAs) that are exported to the cytoplasm via a mechanism that involves exportin-5. Dicer, a cytoplasmic RNase, subsequently cleave the pre-miRNAs into ~ 22 nucleotide miRNA duplexes (200). The duplex strand with the highest base pairing stability (the 'passenger' strand) is then degraded, whereas the other (the 'guide' strand) becomes selectively loaded into a microRNA ribonucleoprotein effector (miRNP) and associates with one of the highly conserved Argonaute proteins (199;200). The best characterized miRNP is known as the RNA-induced silencing complex (RISC), and acts as a versatile mRNA-regulating platform to exert different types of regulatory responses.

The miRNA-RISC complex binds to the complementary 3'- or 5' untranslated region (UTR) of target mRNA and regulate protein synthesis by either translation inhibition (when complementary binding is partial) or deadenylation and mRNA degradation (when complementary binding is near-perfect) (195). The first mode of action implies that miRNA may alter protein expression without detectable changes in target mRNA, posing challenges to the widespread use of cDNA-based quantitative PCR. The individual miRNAs seem to have the potential to regulate multiple genes and thus, participate in larger gene networks designed to control specific biological processes (201). In addition to protein coding genes, miRNAs may target promoters, regulate the transcription factors directly, or repress genes that act as negative regulators of other genes, thus indirectly upregulating a specific target

gene (202). Through this organization, a specific miRNA is capable of effectuating widespread genetic regulation and elicit large functional changes within the cell.

However, multi-level control of the miRNA pathway complicates the efforts to clarify the functions of individual miRNAs. For example, comparison of tumor-specific mature miRNA expression with pri-miRNA transcript levels showed poor correlation and suggest that maturation is a regulated process (195). Stabilization of the cytoplasmic Dicer through MAPK/ERK-mediated phosphorylation, epigenetic mechanisms such as DNA methylation and selective usage of alternate 3'UTR isoforms by target mRNAs also represents various modifiers of the system (195;200). Our limited understanding of such miRNA-pathway regulatory mechanisms is bound to hamper attempts at *in silico* algorithmic prediction of specific miRNA effects for the time being, and does in all likelihood result in significant false positive and negative discovery rates when trying to predict miRNA-mRNA interactions by computational modeling alone. Experimental confirmation is therefore vital when a predicted miRNA-mRNA interaction is put forth.

2.6.3 The role of microRNA in immunity

Emerging evidence on miRNA is currently reshaping our understanding of intracellular immune cell regulation and coordination, revealing a level of molecular control beyond the established roles of signaling pathway mediated by kinases and transcription factors. Systematic analyses have revealed that different hematopoietic organs and cell types exhibit distinct miRNA profiles requisite for correct development, differentiation and stability of several immune cell lineages (198). Experimental evidence suggests that miRNA processes can be influenced by immune challenges, inflammation or other forms of cellular stress. In addition, miRNA have been demonstrated to regulate antigen-presentation, Toll-like receptor signaling cascades and cytokine production, immunoglobulin class-switch recombination in B cells and T cell receptor signaling, as well as proving themselves fundamental players in B and T cell development and differentiation (198;202). Signal transmission through miRNA-containing exosomes, which are specialized membranous nano-sized vesicles derived from endocytic compartments, have been proposed as a well suited mode of signal transmission when controlling space-confined processes such as the initiation of the immune reponse (203).

Dysfunctional miRNA expression has been associated with a wide variety of human diseases, including both acute and chronic inflammation as well as viral infections. A recent paper by Sonkoly and Pivarcsi (195) suggests four possible routes miRNA may contribute to or cause diseases;

I. Loss or downregulation of miRNA expression due to mutation, epigenetic inactivation, aberrant processing or transcriptional down-regulation, allowing for uncontrolled expression of their targets
II. Overexpression of a miRNA due to gene amplification or mutations in its promoter region or due to transcriptional upregulation, causing suppressed production of target proteins
III. A mutation in the 3'UTR of an mRNA may affect a miRNA binding site and the miRNA may no longer be able to bind to it, resulting in overexpression of the target mRNA
IV. A mutation in the 3'UTR of a gene may generate a new miRNA binding site, resulting in unpredictable genetic effects.

Interestingly, miRNAs encoded in the viral genome are capable of reshaping the cellular environment to maximize viral replication, hijacking the host cell miRNA machinery for its own malicious purposes. Use of miRNA has numerous advantages from a viral perspective: they can specifically down-regulate host cell genes, they occupy less than 200 nucleotides of the viral genome and they lack antigenic properties (195). For example, the presence of the liver-specific miR-122 is a prerequisite for successful hepatitis C virus (HCV) replication, and the Epstein-Barr virus (EBV) uses exosomes to transmit EBV-encoded miRNAs from infected to non-infected cells, including monocytes and T-cells (198;203).

A successful inflammatory response to infectious stimuli necessitate the induction and coordination of several hundred genes aimed at achieving pathogen clearance, while at the same time maintaining tight control of the proinflammatory cascades to prevent the process from escalating into run-away inflammation. Expression profiling of 200 miRNAs in the human monocytic THP-1 cell line revealed miR-146, miR-132 and miR-155 as endotoxin-responsive genes (204). Cell surface TLRs (TLR2, TLR4 and TLR5) all induced miR-146, while intracellular TLRs (TLR3, TLR7 and TLR9) did not elicit a similar effect. The proinflammatory cytokines tumor necrosis factor α (TNFα) and interleukin-1β (IL-1β) also upregulated miR-146 expression via the NF-κB pathway. Of note, miR-146 was found to target two key adaptor proteins in the TLR/ IL-1β pathway, namely TNFR-associated factor 6 (TRAF6) and IRAK1, suggesting that miR-146 may function as a negative feedback mechanism regulating bacterial-induced TLR-signaling (204). Further studies in THP-1 cells have revealed miR-146 to rise 4 hours after endotoxin challenge, increasing to a 35-fold upregulated expression over 24 hours (205). This could be related to monocytic lipopolysaccharide (LPS) tolerance, a phenomenon where cells that have been given an LPS-priming dose exhibits less responsiveness to new challenges for a time period. LPS-tolerant THP-1 cells were observed to regain responsiveness, as measured by TNFα production, when miR-146 decreased to normal levels after 22 hours. The same study demonstrated that

transfection with miR-146 mimicked LPS-priming, while transfection with a miR-146 inhibitor disrupted normal endotoxin tolerance (205). This mechanism also appears to be responsible for the cross-tolerance developed for non-LPS TLR-ligands after priming with LPS, and seems reproducible when using other bacterial compounds such as peptidoglycan as the priming agent (206).

In contrast to miR-146, miR-155 is strongly induced by antiviral response cytokines such as interferon-β (IFN-β) and interferon-γ (IFN-γ) (204). In T-cells, miR-155 expression has been found to be under control of the CREB transcription factor, via Fox3p, and can repress several genes in the LPS signaling pathway (FADD, IKKε and Ripk1) as miR-155 targets (207;208). Fox3p induction by the cAMP signaling system has also been shown to repress expression of miR-142-3p, which targets the adenylyl cyclase 9 (AC9) isoform (209).

Another miRNA, miR-125b, have been found to be repressed by LPS-treatment both in the Raw 264.7 cell line and in primary human macrophages (207;210). This miRNA target the κB-Ras2 3'UTR, an inhibitor of NF-κB signaling, and thus, represses TNFα production. miR-125b have been proposed as a switch that might ensure that the LPS pathway is turned off in the absence of microbial stimuli, while an LPS-mediated downregulation allows for the initiation of the inflammatory response when needed.

Unfortunately, considerably less work focusing on the roles of miRNA in innate immune responses has been conducted *in vivo*. Using the technique of bronchoalveolar lavage on mouse lungs to harvest neutrophils after aerosolized LPS exposure, miRNA profiling demonstrated a rapid increase (peaking at 1 hour) in twelve miRNAs (miR-21, miR-25, miR-27b, miR-100, miR-140, miR-142-3p, miR-181c, miR-187, miR-194, miR-214, miR-223 and miR-224) (211). Microarray profiling of mice injected with LPS intraperitoneally found 19 miRNAs to be upregulated (among them miR-21, miR-146 and miR-155), with special attention being paid to miR-1224 targeting of Sp1 mRNA, whose coding product is a transcription factor that regulates TNFα (212). In a model of radiation-induced thymic lymphoma, TLR4 knock-out mice has substantially lower levels of miR-21 and miR-155, which coincided with a reduced inflammatory response (213).

In humans, the potential of miRNA as biomarkers for disease processes have been proposed, taking advantage of the fact that exosomal secreted miRNA is very stable in plasma. Indeed, an example of such use was illustrated recently by a study demonstrating that serum miR-146 and miR-223 may predict sepsis with both high specificity and sensitivity (214).

Over the last decade, it has become clear that miRNA are deeply involved in regulating the network of inflammatory genes that lie at the heart of sepsis. Our understanding of how this added layer of regulatory complexity interacts with the mediators of the innate immune system is still in its nascency. However, the study of miRNA is a field marked by rapid development, with increasingly precise predictions using software algorithms on their genetic structure. Thus, the discovery of miRNA offers a new avenue of therapeutic approaches, and could have the potential to substantially improve survival rates in sepsis.

3. Aims of this work

- Characterize the effects of LPS on AC isoform expression *in vivo, ex vivo* and *in vitro* in order to explore whether previously observed reductions of cAMP levels in sepsis are caused by reduced expression of the production enzyme.
- Investigate the ability of the cAMP signaling pathway to exert regulation of TNFα protein release.
- Examine whether the effects of LPS-injection *in vivo* can be replicated using an alternate experimental sepsis animal model, the cecal ligation and puncture procedure.
- Explore the molecular mechanisms leading from inflammatory activation (endotoxemia, sepsis) via AC isoform attenuation to reduced proinflammatory mediator production.

4. Summary of results

Paper I

We hypothesized that lower levels of cAMP during sepsis was caused by a depression in adenylyl cyclase expression, and on the grounds of available publications detailing isoform specificity in other cell populations, we wanted to examine if such was also the case in endotoxemia. We mapped the mRNA expression of AC isoforms in major organ systems in the rat, and also explored the effects mediated by 6 hours of LPS infusion on AC expression levels. In the endotoxemic rats, our observations indicated a general depression of AC mRNA expression, presumably reducing cAMP production capability. In many samples, the size of the effects was discernible. Spleen, the most richly vascularized organ we isolated, showed a near obliteration of its AC expression. AC6 was widely down-regulated in many organs, including heart and liver, but not in lung or liver macrophages. In rat liver macrophages/Kupffer cells, we demonstrated that prostaglandin E2-mediated cAMP production was inhibited by LPS treatment, in line with a marked attenuation of AC4, AC7 and AC9 expression. Paper I was the first to report that LPS may downregulate expression of the cAMP-producing enzymes in vivo.

Paper II

In paper II, we sought to explore whether the attenuation of AC mRNA isoforms observed in an endotoxemic model could be reproduced in a cecal ligation and puncture model, as the latter is generally considered a closer approximation of clinical sepsis. 18 hours after the CLP procedure we found reduced expression levels of several AC isoforms in kidney, spleen and liver. The AC9 isoform, which was downregulated in all three tissues, has previously been reported to be regulated by the microRNA miR-142-3p in T-cells. We hypothesized that this regulatory mechanism may be conserved across immune cells, and when mapped the expression, we found miR-142-3p expression to be elevated in kidney, spleen, and liver, as well as in liver endothelial cells. In order to examine whether the inverse correlation between miR-142-3p and AC9 was causally related, we transfected Kupffer cells with miR-142-3p knockdown siRNA and subsequently stimulated with LPS after 24 hours. We observed that LPS treatment increased miR-142-3p and reduced AC9 expression levels in Kupffer cells in vitro. After transfection, the rise in miR-142-3p and corresponding drop in AC9 mRNA expression was abolished, indicating that miR 142-3p may be part of the casual pathway from LPS recognition to AC9 isoform attenuation.

Paper III

We have found a notable gender difference in LPS-mediated TNFα responses in human whole blood, showing significantly higher peak levels (6h after LPS) for young male donors (20-30 years), and sought to examine if cAMP-mediated TNFα regulation could play a role. Several cAMP-elevating agents (8-CPT-cAMP, rolipram, isoproterenol and PGE2) all strongly inhibited the TNFα response in whole blood after LPS exposure. Based on the findings in macrophages/spleen in Paper I, we hypothesized that there may be significant AC regulation by LPS also in human monocytes. We observed that the calcium-activated AC isoforms AC4 and AC7 showed the highest expression levels in monocytes, and both were strongly attenuated after 3 hours of LPS exposure in males only. AC6 and AC9 were also expressed in human monocytes, and the same male-specific trend was observed for AC9, but not AC6. Through siRNA knock-down experiments in the human monocyte cell line THP-1, we demonstrated that AC7 and AC9 knock-down led to significantly higher LPS-mediated TNFα release, which was not observed for AC4 and AC6. The data indicate that male-specific inhibition of AC7 and AC9 may play a role in gender-differences in TNFα release.

5. Discussion

5.1 Methodological considerations

This section will discuss some of the general characteristics and pro et contra considerations of the models chosen in the present work. For a complete reference of detailed protocol, technical data or suppliers, please refer to the corresponding sections of Paper I-III.

5.1.1 Experimental animal models

In order to explore how AC isozyme expression changed in sepsis, we employed the two of the most commonly used animal models of experimental systemic inflammation, the endotoxemia model (Paper I), and the cecal ligation and puncture model (Paper II). These models are most commonly used in rodents; rats and mice. In general, the advantage of using the murine model is the possibility to study knock-out animals, whereas the size of a rat simplifies tissue analysis and cell isolation. In the endotoxemia model, the animals receive an intravenous injection of LPS over a ten minute period. Acting through the TLR4 pathway, LPS is able to reproduce most of the pathophysiological features of sepsis, causing hemodynamic collapse and multi-organ failure within 6 hours (215;216). Infusion in the central vein of a sedated animal provides for a controlled induction of the inflammatory mechanisms leading to multi organ failure and shock in a model which is widely used and validated internationally. By choosing endotoxin as our model agent, we ensure that the inflammatory response runs down-stream of the most meticulously characterized system, the TLR4 receptor. The endotoxemic model also has the advantage that the infectious stimulus is directly quantifiable and conditions are easy to standardize.

The most commonly used alternative experimental sepsis model to endotoxemia is the cecal ligation and puncture (CLP) procedure. Since its conception by Chaudry in 1979, more than one thousand scientific articles have been published using the procedure for research into septic shock (217). Using an abdominal incision, the cecum is ligated and punctured by an 18” G needle, facilitating a small droplet of feces into the abdominal cavity. Mimicking a ruptured appendix, the animals then develop peritonitis, sepsis, organ dysfunction and shock over the period of 18-24 hours. As such, the model is much closer to the clinical picture of human sepsis, which generally develops more insidiously than what can be seen in the rapid disease progression in response to LPS injection. The CLP operation produces a polymicrobial active infection, as opposed to the use of a single, non-living agent used to artificially induce shock in endotoxemia. Its noisier, but clinically more realistic picture is an advantage when testing the effectiveness of therapeutic interventions in animal trials. An added

advantage is the potential to run longer studies with hard end-points, something precluded in the sedated endotoxemic animal.

Other models exist, but are less frequently used. Urosepsis has been modeled in a model with uterine ligation and inoculation of E. coli (218). Some authors also use a version of the procedure where antibiotic treatment is added (219).

Neither of the models are precise representations of the human condition, which may help explain the preponderance of beneficial interventions in experimental animal research within the field, as benefits repeatedly fail to be reproduced in human clinical trials. Both models have a place in sepsis research, but results need to be interpreted with an eye for where the models resemble human clinical disease and where they do not. We believe both models have value in exploring the molecular mechanisms underlying the pathophysiological process in sepsis, and found it worthwhile to examine AC expression profiles in both models.

5.1.2 The whole blood model

The simple *ex vivo* whole blood model represents a good choice for introductory exploration of circulating immune cells and their extracellular signaling. Its simplicity and robustness allows for a wide range of experiments on the cytokine responses in a mixed white blood cell population, while the experimental set-up allows for quick expansion to a large number of research subjects if an initial pilot produces interesting observations. The model is characterized by stable leukocyte count and high viability (220). The endogenous presence of soluble factors like LBP and soluble CD14 in plasma facilitates and potentiates LPS detection by the cells of the innate immune system, with the former protein having a concentration-dependent dual role which promotes LPS-induced activation at low concentrations while it tampers LPS-stimulation when involved in the acute-phase response (221). From our vantage point (Paper III), another advantage with the model was the presence of endogenous levels of gender hormones at the time when the blood was incubated with LPS. Both the TLR4 and cAMP pathways could easily be stimulated at various steps in all the leukocytes subject to study, and their effects on inflammatory mediator release be examined (222). The whole blood model thus represented a fast and inexpensive way to study how LPS interacted with AC production capacity, and how different cAMP levels influenced TNFα release.

5.1.3 Macrophages and monocytes

Inflammation is generally initiated by the recognition of an infectious pathogen by the local immune defense system, chiefly in the form of contact with resident tissue macrophages. Although present in

every organ, the liver plays a particularly important role in the innate immune system *in vivo*, illustrated by its disproportionally abundant presence of macrophages. About 60-80 % of the total human resident macrophage population resides in the liver, where they line the narrow sinusoids and constitute 15 % of the total liver cell population (223-225). By surveying the bloodstream filtered through the organ, they are positioned to instigate the systemic inflammatory process upon recognition of infection and tissue damage. In the clinical case of a ruptured appendix, Kupffer cells will react by sending out a massive call for reinforcements by releasing cytokines and chemokines into the circulation. Chemokines allow circulating leukocytes to travel towards the originating site, and cytokines activate the cells for defusing the pathogen. Kupffer cells have been shown to be key players in initiating the shock syndrome in the CLP model (60). On this basis, we isolated primary Kupffer cells isolated after liver perfusion and cultured them for further experiments.

Monocytes and macrophages share many structural and functional similarities, as they represent different stages in the cell's development. We suspected that the regulatory cAMP pathway may be conserved across innate immune cells, and hypothesized that monocytes would be subject to similar regulation as macrophages. As monocytes are more readily available than tissue macrophages in humans, this allowed us to study human AC regulation. Combined with the simple set-up of the whole blood model, the isolation of monocytes using magnetic beads allowed us to gather samples for a substantial number of donors. Monocytes also make an interesting object of study in themselves, since the production of TNFα in LPS-stimulated *ex vivo* blood can primarily be prescribed to monocytes (226).

5.1.4 mRNA instead of protein

It is a limitation that a considerable proportion of our work consists of mRNA data without verification through protein expression. At various point during the course of our work, we have attempted to visualize the AC and cAMP pathway proteins and their interactions using fluorescence microscopy, Western blot or other protein-based methods. Unfortunately, immunodetection of adenylyl cyclase proteins is difficult both due to low levels in most cells (0.01–0.001% of membrane protein), and the lack of high quality antibodies (133;227;228). In fluorescence and confocal microscopy, the antibodies gave consistently weak signals, and we were unable to produce high-quality images due to autoflorenscence from surrounding lipofuscein in the Kupffer cells, despite repeated attempts at various quenching protocols. Although we managed to identify bands on Western blots, the results were either diminishingly weak or at unexpected protein lengths, causing us to conclude that the antibodies at our disposal were not good enough for visual studies of the

behavior of the AC isozymes. For these reasons, we had to look for predicted functional results of protein regulation rather than the proteins themselves in order to complement our mRNA observations.

5.2 Adenylyl cyclase regulation in monocytes/macrophages and the inflammatory response

5.2.1 Attenuated AC expression in monocytes and macrophages: Role in sepsis/endotoxemia?

As elaborated upon in the introductory section, there is an ample literature underscoring how intracellular cAMP signaling is a sinewy regulator of inflammatory responses in monocytes and macrophages. In the present work, we explored LPS-mediated regulatory effects on AC expression in rat Kupffer cells and alveolar macrophages, as well as in human *ex vivo* monocytes.

In paper I we reported that rat Kupffer cells isolated after 6 hours of endotoxemia were inhibited with regards to AC expression, with the isoforms AC4, AC7 and AC9 all being significantly attenuated compared to cells from sham-operated animals. In paper II we found similar AC attenuation in Kupffer cells 18 hours after being subjected to a polymicrobial model, with reductions in AC4, AC5, AC6 and AC9. Rat alveolar macrophages, on the other hand, had less pronounced alterations in response to LPS and AC5 was the only isoform where downregulation was statistically significant. The pathological process of endotoxemia is one defined by massive release of proinflammatory mediators, followed by rapid progression of systemic inflammation and organ injury in the course of a mere 6 hours (229). In contrast, the course of sepsis induced by the CLP procedure is markedly slower, with organ injury markers rising later and more gradually (230).

From our data it is not possible to draw definite conclusions as to whether the observed differences between the results are due to the characteristics of the model, differences in the cell populations or temporal aspects of the sampling. However, both experiments indicate that the reduced levels of cAMP in peripheral blood monocytes, shown to be a feature of both septic patients and healthy donors exposed to septic serum, may be caused by impaired production of cAMP by adenylyl cyclases (180;181). To explore this possibility (Paper III/Manuscript I), we exposed human full blood to LPS ex vivo for 3 hours before isolating monocytes. In male donors, we observed significant reductions in monocyte AC4 and AC7, as well as a trend ($p = .048$) towards attenuation of AC9. No such statistically significant association was evident in females, and a few (2-3 out of 17 for each AC isoform) even exhibited a 4-8 fold increase.

As discussed in the introductory section, an important attribute of the cAMP second messenger system is its capability of delivering a precise, spatially confined signal through subcellular compartmentalization (95). The individual roles of the AC isoforms are poorly characterized in immune cells, but their different regulatory properties may provide clues. For example, PGE2 and forskolin differ in their capacity to activate AC9, which the latter fail to induce (134). We found that pretreatment with LPS had discriminatory effects on the stimulatory power of these agonists, a finding which has also been shown by others (231). This finding suggests a role for AC9 in PGE2-mediated signaling. In a trial of administering marrow to sepsis patients, PGE2 was recently ascribed a key role in stimulating monocyte and macrophage IL-10 production (232). Immunofluorescent techniques have identified a pathway from PGE2 through EPAC1 which colocalized with micro-tubuli organizing centers (MTOCs) and mediated FcR-phagocytosis (159;233). Macrophage levels of cAMP has been closely implicated as core regulators of their internal physiology, and manipulation of cAMP can transform classically activated (M1) macrophages to resolution-phase macrophages (rM) when levels are heightened, et vice versa (234).

From previous work in our lab, we know that AC9 is highly expressed in Kupffer cells, and that cAMP signaling can attenuate TNFα release (134). It was also recently demonstrated that mice deficient in AC7 have macrophages that generate a weaker cAMP response to inflammatory stimuli and respond to LPS challenges with increased TNFα release, causing them to become hypersensitive to endotoxemic shock (235). In THP-1 cells, knock-down siRNA directed against AC7 and AC9 expression effectively doubled the TNFα release in response to LPS, while transfection with AC4 and AC6 did not (236). There were also indications that the PDE4-selective inhibitor rolipram had diminished anti-inflammatory effect (Supplemental figure), which would be consistent with impaired production capacity in the subcellular isoform-specific cAMP pools.

In summary, we find that our experiments corroborate earlier observations by our own group and others underscoring the importance of the AC7 and AC9 isoforms as contributors to inflammatory mediator release. As far as we know, we are the first to demonstrate the effects of both AC7 and AC9 on TNF-α release in a human monocytic cell line

5.2.2 miR-142-3p and AC9 downregulation

The decrease in AC9 expression observed in the CLP model (Paper II), led us to explore the expression level of miR-142-3p in organs and the main liver cell populations in the aftermath of CLP (237). At 18 hours, we found significant increases in the liver, kidney and spleen that correlated with reduced expression of AC9 mRNA. This was also true when we examined miR-142-3p and AC9 in Kupffer cells

and LSEC cultures. Based on these findings, we hypothesized that AC9 mRNA and protein expression was downregulated by miR142-3p in innate immune cells, analogous to the regulatory pathway observed in T-cells. When we transfected primary rat Kupffer cells with miR-142-3p antagomir, we found this manipulation to effectively prevent the LPS-mediated AC9 attenuation otherwise witnessed in control cells transfected with scrambled siRNA (238;239).

MicroRNA, virtually unknown merely a decade ago, swiftly evolved from scientific curiosity to the point where it is shifting our paradigms of genetic regulation. They have proved themselves to be crucial players in a wide range of cellular functions, and the experimental evidence indicate that they exercise key functions in facilitating normal immune responses as well. Although the emergence of microRNA-mediated regulation will not make the understanding of intracellular signaling networks any easier, they do provide new avenues of approach for diagnosis and therapy. Given our current knowledge, it seems a safe prediction that the use of microRNA for clinical applications will rise quickly in the coming years, and that targeting of disease-related microRNA has the potential to give rise to radically new treatment principles for a wide array of pathological conditions.

As microRNA is considered to be an evolutionary ancient component of genetic regulation with high conservation rates, we hypothesized that some functions might also be conserved across different populations of immune cells (197). AC9 had recently been shown to be highly expressed in regulatory T-cells, and appear linked to their suppressive function (240). The same study demonstrated that miR-142-3p target the 3'-UTR (untranslated region) of AC9, and thus lead to reduced expression levels of isozyme mRNA and protein. All hematopoietic cell lineages have been found to have abundant expression of miR-142-3p, where the microRNA appears to be preferentially expressed (241).

Several microRNAs have been implicated in inflammatory diseases, and elevated levels of plasma microRNA have previously been associated with liver injury and sepsis (214;242-244). In diseases were cAMP activity has previously been shown to be dysregulated, such as psoriatic skin and multiple sclerosis patients, other investigators have found expression of miR-142-3p to be elevated in inflammatory phases, implicating it as a potential pathological agent or intermediary that could led to loss of appropriate AC9 function (242;244).

Since our experiments, others have found miR-142-3p to have important roles in myeloid differentiation, directly inhibiting cyclin T2 and TGF-β activating kinase binding protein 2 genes, and decreased cAMP levels with weakened PKA activity in response to increased miR-142-3p has been reproduced in primary cells from acute lymphoblastic leukemia patients (245;246). Meanwhile, it has

also been reported that IL-10 knock-out mice have higher baseline miR-142-3p expression in circulating leukocytes, with increasing miR-142-3p levels corresponding with worsening inflammatory pathology resulting from the lack of IL-10 (247). Exogenous IL-10 exposure failed to restore miR-142-3p levels to normal levels. A study published earlier this year found that peripheral blood mononuclear cell content of miR-142-3p correlated with operational tolerance, and proposed the existence of a negative feedback loop involving miR-142-3p to maintain this tolerance (248). The same study overexpressed miR-142-3p in the Raji B-cell line, where it was discovered to regulate almost a thousand mRNA transcripts, of which 49 have known roles in inflammation.

At the same time as we conducted our experiments, another group examined the effect of miR-142-3p on IL-6 in mouse dendritic cells and reported the somewhat conflicting finding that IL-6 seemed to rise with miR-142-3p knock-down (249). However, when performing in vivo transfection with miR-142-3p, TNF-α mRNA and protein expression were both significantly reduced in wild-type mice subjected to antagomir, and silencing miR-142-3p also improved overall survival in response to endotoxemia.

Taken together with the previously discussed differential role of the AC9 isozyme in the regulation of proinflammatory TNFα and anti-inflammatory IL-10, we conclude that an increase in miR-142-3p after CLP appears to lead to a reduction of AC9 and weakening of spatially relevant cAMP production in macrophages, tilting the innate immune system in a direction of aggregated and inappropriate proinflammatory response (234). In the context of the initial proinflammatory phase of severe systemic inflammation, we therefore propose that high levels of miR-142-3p in innate immune cells may be associated with more severe outcomes. The cAMP pathway regulates anti-inflammatory polarization of macrophages to healing and reparation during the secondary response, and miR-142-3p may have a role in reversing this.

5.3 Potential implications of AC regulation in different tissues

5.3.1 Attenuation of AC expression after LPS-exposure

In paper I, one of the main findings was a marked reduction in the mRNA levels of most adenylyl cyclase isoforms after 6 hours of endotoxin infusion in the rat, with some indication of differential regulation in various organs and cell populations. Within this time frame, the animals developed multiple organ damage, as assessed both morphologically and by markers of liver and kidney injury (60). For paper II, we hypothesized that AC expression would also be attenuated in a model of bacterial infection and sepsis. The CLP model is intended to mimic the clinical situation of a septic

peritonitis, with markedly slower progression than the clinical course caused by infusion of LPS. Still, systemic inflammation and organ injury (as determined by plasma values of commonly used biochemical markers) was decidedly evident at both 10 and 18 hours (237). We found that the changes in AC mRNA expression from CLP sepsis did resemble those observed in endotoxemia, giving the impression of a broad and widespread downregulation of the cAMP producing isozymes.

As we have already pointed out, there are numerous studies demonstrating that global cellular levels of cAMP are reduced in response to endotoxemia and sepsis both among animals and humans (175-178;180;181). The widespread attenuation of AC isozyme expression after LPS injection or exposure to a polymicrobial infection observed by us would provide a plausible cause for the decreased cAMP levels described by others. Unfortunately, knowledge of the specific roles of individual ACs is scarce in many tissues or cell populations; however, substantial advances have been done within heart and endothelial barrier research with regards to our understanding of this system. In this section, we will review and discuss the literature with regards to the potential implications of the observed reduced cAMP production capacity in select tissues.

5.3.2 AC isoform regulation in the heart

In paper I, we found the mRNA levels of AC6 to be significantly down-regulated in heart tissue from endotoxemic animals, with mean expression levels close to a quarter of the sham operated group (215).

The cAMP system has an important role in the heart; not only does it regulate the heart rate by mediating external catecholaminergic signals, but it also regulates the functional response to a plethora of other hormones and neurotransmitters (96). Cardiac myocytes primarily express two isoforms of ACs, AC5 and AC6 (250). While AC6 is expressed in most tissues at a low level, AC5 appears to be most abundant in only two; brain and heart (251;252). There are indications that the isozymes are developmentally regulated in an opposite manner, with AC6 declining gradually with age from high fetal levels and AC5 following a complementary pattern (253;254). Through a series of knock-out investigations, their roles are gradually being elucidated and disruption of spatial control of the signal seems crucial in the development of heart pathology (96;255;256).

Experiments indicating that failing myocardium have reduced amounts of basal cAMP and an impaired AC functionality in response to agonist stimulation (257;258). AC5 appear to be crucial for normal heart functions, with experimental induced deficiencies producing deleterious effects, while a reduction of AC6 mRNA in the failing ventricle precipitate a drop in left ventricle (LV) contractile

function (251;254;255;259). Cardiac-directed expression of AC6 in transgenic mice shows no increases in baseline heart rate and contractile function or detectable abnormalities at advanced age, despite a 20-fold excess of protein. However, hearts exhibited a marked increase in heart rate and contractile function in response to βAR stimulation, in line with previous *in vitro* reports from the same group indicating AC (and not βAR) as the rate-limiting step in cAMP generation (260;261). *In vivo* studies of mouse models of acute myocardial infarction and cardiomyopathy associated AC6 expression with increased LV contractile function, reduced mortality and reduced incidence of high-grade atrioventricular (AV) node block (262-265). Indeed, AC6 has been shown to be expressed in the AV node and facilitate nodal anterograde and retrograde conduction without altering sinus node conduction (266). Last year, it was reported that a tet-regulated AC6 overexpression model activation exerted beneficial effects even when the gene was turned on five weeks after the establishment of severe chronic heart failure (CHF), subsequently improving function of the failing heart (267).

There has been some speculation in the cardioprotective mechanisms of AC6 overexpression (227). In cardiac fibroblasts, AC6 was recently demonstrated to be completely compartmentalized in lipid raft domains where it was activated solely by coresident GPCRs (268). A similar situation may be present in cardiac myocytes. Expression of AC6 through adenovirus-mediated gene transfer to cultured myocytes and through an *in vivo* cardiomyopathic setting has suggested a decrease in phospholamban (PLB) expression, possibly by marked upregulation of activating transcription factor 3 (ATF3) (269). ATF3, a transcriptional suppressor, binds to and inhibits the PLB promoter (270). However, AC6 expression was also associated with increased phosphorylation of both PLB and Akt (227;269). Recently, a report describing AC6 knock-out mice showed that although basal cAMP levels were normal, there was a markedly decreased responsiveness to cAMP-elevating agents such as isoprotrenol, forskolin and the forskolin-derivative colforsin, and AC6 deletion also impaired Ca^{2+}-mediated inhibition of AC activity. The disruption of normal AC6 expression also proved to have profoundly negative effects on LV calcium handling, with significant reductions in PLB phosphorylation and SERCA2a activity, and conspicuous abnormalities in calcium transient formation by caffeine stimulation was interpreted as evidence for marked impairment of SR calcium storage capacity (271).

As these changes are predicted to increase intracellular calcium availability and LV contractile function, the LPS-mediated attenuation observed in heart AC6 mRNA levels after 6 hours of infusion could be presumed to be correspondingly detrimental to heart function in shock (269). In addition, there is a close scientific linkage between βAR (and hence, sympathetic) responsiveness and level of

AC6 mRNA in cardiomyocytes, which could be hypothesized to underlie the clinical observation of desensitation to vasocontricting drugs during intensive care treatment for septic shock (3;227).

5.3.3 AC and cAMP in the maintenance of endothelial integrity

In our initial paper (Paper I) we observed widespread LPS-mediated down-regulation of AC6 mRNA expression in several richly vasculated organs (215). These findings spurred more specific examination of this isozyme in Paper II, where we examined cultures of isolated liver sinusoidal endothelial cells (LSEC) levels 18 hours after LPS stimulation and found AC6 to be approximately 26% of the level in control cultures (237).

The endothelial cell (EC) layer forms a semi-permeable dynamic barrier between the vascular space of blood vessels and underlying tissues. Under normal conditions, ECs tightly adhere to each other and interact only fecklessly with the cells circulating in blood. Upon exposure to inflammatory mediators, a disassembly of adherence junctions occur, the endothelial cells reversibly rounds up, and the expression of surface receptors that interact with circulating leukocytes is induced (272).

This change in the endothelium allows transendothelial migration of leukocytes, and is also responsible for the extravascular leakage in septic shock. In addition to its previously discussed ability to attenuate the expression of adhesion molecules, a heightened cAMP level is demonstrated to play an important role in maintaining the endothelial barrier. The inflammatory changes in endothelial structure and function are oppositely regulated by two intracellular signaling mediators, calcium and cAMP. Intracellular calcium promotes the disassembly of adherence junctions and extravascular leakage, whereas cAMP signaling preserves the junctions (273).

Notably, the adherence junctions in the endothelium are shown to be contained by cAMP-dependent mechanisms in an AC6-specific manner, mediated by both PKA and Epacs (122;274;275). PKA appears to have the most central function in inhibiting several of the calcium-mediated effects, including the stress fiber formation leading to morphological changes, and the translocation of focal adhesion proteins involved in gap formation (273). The AC6 isoform is sensitive to calcium-mediated inhibition, which may explain why intracellular calcium disturbs cAMP-mediated protection of the endothelial barrier (276). The protease thrombin, which is activated in septic shock and induces blood coagulation, is a particularly potent inducer of interendothelial gap formation (272). The endothelial protease-activated receptor of thrombin (PAR1) not only induces calcium, but also directly inhibits AC activity by releasing inhibitory G-protein (277).

It is important to note that liver sinus endothelium differs from vascular endothelium. LSEC form a fenestrated monolayer at the inside of the sinusoids, acting as a barrier between the blood flow and the perisinusoidal space (278). The open pores of the LSEC fenestrae makes permeability high compared to other microvascular endothelial cells, and facilitate the flow of soluble macromolecules from the circulation for hepatic clearance. Unfortunately, primary LSEC are not amenable to long-term culture and lose their phenotype within days after being extracted (279). The junctional complexes between adjacent LSEC cells are of a distinct molecular composition, consisting of a mix of junctional proteins shared with other endothelial populations and novel proteins reserved for LSEC alone (278-280). However, the first comprehensive analysis of its molecular structure published earlier this year concluded that human LSEC assemble the same molecular complexes typical of endothelial adherens junctions, and abundantly express the main transmembrane component, VE-cadherin, on both mRNA and protein levels at similar locations as other endothelial cells, disproving the notion that LSEC lack interendothelial junctions (278). LSEC also express all members of the family of junctional adhesion molecules, but human cells lack expression of the occludin protein commonly found in tight junctions (278).

Both Paper I and II suggest that LPS has the potential to cause widespread AC6 attenuation across multiple organs, including the observed reduction of AC6 expression levels of liver endothelium. If additional subpopulations of endothelial cells could be confirmed to respond in the same way to LPS, this would suggest that the loss of endothelial integrity, increased permeability, extravascular leakage and ultimately, hypotensive shock, may be due to AC6 down-regulation induced by LPS.

5.3.4 Reduced AC expression in spleen

The most conspicuous reductions in mRNA expression for all AC isozymes except AC5 were observed in spleen after LPS infusion (215). With the CLP procedure, a marked drop could be measured in the two isoforms most closely associated with immunological effects, AC7 and AC9 (237).

Unfortunately, earlier inquiry aimed at understanding the cAMP signaling system in spleen has been extremely sparse. However, possible implications may be speculated upon on the basis of the physiological role of the spleen itself. The spleen is an immunological and hematological organ, and contributes significantly to the production of pro- and anti-inflammatory cytokines (281). For example, splenectomy results in dramatic attenuation of the normal IL-6 response, making individuals susceptible to blood-borne bacterial infections, and in vitro measurements of lymphocyte signaling responses indicate that the normal CREB, EKR1/2 and p38 downstream response is absent (281). The spleen is also a reservoir of various immune cells responsible for innate and adaptive

immunity, boosting their numbers in circulation during the infectious response. That splenectomized individuals are at a higher risk for myocardial heart disease decades later, underscores the important role of the spleen in normal immunological homeostasis (282).

The denotative obliteration of all identifiable ACs besides AC5 strongly suggest a response in immune cells, as the tissue harvesting was performed without perfusion and these constitute a major component of the baseline spleen composition. As the cells of the immune system share a wide spectrum of common evolutionary features, part of the response may also be due to regulation of the large population of B- and T-lymphocytes. In both the endotoxemia and CLP experimental models, AC9 expression levels were markedly reduced compared to sham. AC9 has been reported to be specifically involved in polarization of T-cells towards regulatory T cells (283), which would also imply the suppression of T-cell proinflammatory cytokine inhibition. Together with AC7, attenuated levels of both isozymes may suggest that normal anti-inflammatory macrophage polarization is being inhibited.

cAMP signaling not only modulates inflammatory cytokine release from macrophages, but also serves as an inhibitory factor in a wide range of inflammatory responses, including the inhibition of leukocyte-endothelial interactions, neutrophil transmigration and degranulation, cytotoxic responses of NK-cells and T-cells, platelet aggregation and the maintenance of gap junctions. Thus, the dramatic fall in rat spleen AC expression 6 hours after LPS infusion indicated that blood cells had lost substantial cAMP production capability, and that the systemic pro-inflammatory process had spiraled out of control.

5.3.5 Reduced AC expression in liver

The narrow sinusoids of the liver are important areas of host defense against systemic infection, as the dominant population of stationary tissue macrophages reside in the liver as Kupffer cells (284). The sinusoidal endothelium is characterized by being particularly “sticky” to leukocytes and independent of a functional leukocyte rolling step for adhesion, in addition to being discontinuous with fewer tight junctions compared to vessel endothelium elsewhere (285). These features, together with the fact that activated KC and LSEC are very efficient producers of leukocyte-attracting chemokines, make the sinusoids particularly prone to leukocyte accumulation (286). Liver also contain a large population of resident NK-cells (287).

Although hepatocytes express a number of PRRs, they did not appear to be important targets for AC mRNA regulation in the sepsis model, as no significant differences in AC expression were found in

pure hepatocytes isolated from CLP and sham animals. No significant changes were found in the LSEC cultures, although there was a tendency towards a shift in AC expression from calcium-inhibited isoforms (AC5, AC6, AC9) in cells from sham animals to calcium-insensitive isoforms (AC4, AC7) in cells from CLP, which is interesting, since calcium and cAMP signals together control endothelial permeability under inflammatory conditions, as discussed in 5.3.3 (288). As already mentioned, AC6 has been demonstrated to have a critical role in the maintenance of endothelial gap junctions, and the Epac-regulated adhesion protein VE-cadherin is abundantly expressed in the interendothelial junctions (278;289). It is therefore reasonable to speculate that a shift away from AC6 expression may increase the trans-sinusoidal barrier permeability, contributing to the clinical state observed during the systemic inflammatory response syndrome.

5.4 Adenylyl cyclases as a source of gender differences in the inflammatory response

As we explored TNFα release in the ex vivo human whole blood model with regards to cAMP signaling, it became clear that males had a substantially stronger TNFα response than females at both 3 and 6 hours (236). This induced us to examine the AC isoform expression profiles stratified by gender. At 3 hours, AC4, AC7 and AC9 had all undergone considerable down-regulation, compared to no or minimal change in females. No gender-specific TNFα response was observed when examining older (post-menopausal) individuals.

Our observations are in line with a comparatively large study of 154 healthy volunteers aged around 20 to 30 years found males to produce significantly more TNFα, IL-1β, IL-6 and IL-8 in response to a set challenge of 1μg/ml LPS (290). However, others have found no difference (although the endotoxin dose given was 2 ng/ml) or even a stronger pro-inflammatory response among females in a small (30 individuals of 22±1 years) study (83;84). The potential cyclic component of the female immune response indicates that results should be stratified by contraceptive use and stage of the menstrual cycle, although this was inadequately done in two of the studies (83;290;290;291).

A study of fifty subjects of 65 years of age or older with concurrent young (21 to 40 years) controls reported smaller, but statistically insignificant, release of TNFα at 6 hours in young females compared to males, and a substantial rise in TNFα and IL-6 levels in old, post-menopausal women compared to the younger group (292). Both observations are in line with the findings from the present work.

The difference between pre- and postmenopausal women suggests female hormones as a potential cause for the gender differences in AC expression. Estrogen replacement given to postmenopausal

women before intravenous endotoxin challenge attenuated the pro-inflammatory rise in TNFα and IL-6 levels, and published data from younger donors suggest that human circulating IL-6 response is decreased by estrogen (293-297). In contrast to the conflicting reports from human studies, results obtained from experimental animal studies clearly point to a female, estrogen-mediated advantage in sepsis (77;298). We conducted a computer based search on the promoter sequences of the human AC4 gene (Chromosome 14q12) and the AC7 gene (Chromosome 16q12-13), which exhibited the most evident male-specific inhibition. The promoters of both genes were found to contain putative binding sites for estrogen receptor (ER)α, and for the estrogen-induced transcriptional repressor δEF1/ZEB (299;300).

While a homozygote knockout for AC7 is not compatible with life, heterozygote AC7 +/- mice are reported to express reduced levels of AC7 mRNA, with their macrophages producing increased levels of TNFα (301). In line with this, they are also hypersensitive to endotoxemic shock (235). Our experiment with repression of AC7 and AC9 isoforms using siRNA and nucleofector transfections in THP-1 macrophages led to a stronger TNFα release 6 hours after stimulation with LPS. That AC9 expression appears linked to TNFα regulation in monocytes or macrophages has to our knowledge not previously been demonstrated, but the possibility was suggested in a previous study in rat Kupffer cells (302). Taken together, these studies imply that gender-specific regulation of AC expression levels would produce gender differences in the TNFα response, which matches what is seen in our data.

As should be evident by now, the second messenger system modulates more than cytokine release from macrophages; cAMP signaling inhibits leukocyte-endothelial interactions, neutrophil transmigration and degranulation, cytotoxic responses of NK-cells and T-cells, platelet aggregation, and stabilizes intercellular gap junctions (108;303-308). This wide spectrum of downstream functional consequences reminds us that LPS-mediated AC regulation may yet further unexplored mechanisms of action. Further studies will hopefully reveal if and the gender-specific regulation of AC mRNA expression by LPS involves estrogen-regulated mechanisms or other gender hormone-mediated effects at the AC promoters.

5.5 Potential implications for future treatment of sepsis

It has become increasingly clear that the cAMP pathway is intrinsically involved in balancing the immune response, and interest in novel ways of manipulating the subcellular pools has been steadily rising. Two fairly recent reviews discuss the untapped potential of drug discovery by supply-side

regulation through development of isoform-specific AC agonists and inhibitors (309;310). To the list of suggested uses for such compounds – which currently include analgesics without dependence, memory enhancement, male contraceptives, and drugs for asthma and congestive heart failure – we propose to add sepsis. Although efforts are ongoing in identifying selective compounds for regulating the individual AC isoforms, their development is hampered by the high degree of evolutionary conservation between the isozymes (309-311).

The most successful anti-inflammatory cAMP-directed drugs to date are various inhibitors of the PDE isozymes. For a long time, naturally occurring PDE inhibitors like the methylxanthines theophylline and caffeine have been known to express immunomodulating properties (312). Pentoxifylline, or PTX [1-(5-oxohexyl)-3,7-dimethylxanthine], is a methylxanthine derivate and PDE4-specific phosphodiesterase inhibitor that has been shown to decrease transcription of TNFα in endotoxemia, attenuate release of pro-inflammatory cytokines such as IL-1β, -6 and -8, lower the pool of circulating neutrophils, inhibit granulocyte adherence and transendothelial migration, reduce neutrophil degranulation and release of matrix metalloproteinases and ROS; thus, shifting the whole process towards anti-inflammation and restoration (313-318). Thus, it should be no surprise that PTX has been demonstrated to blunt endotoxin-mediated lung injury, liver damage and acute renal failure, and increase early survival in animal models of septic shock (319;320).

However, uncertainty about the right therapeutic windows (with regards to both timing and dosage) and side effects limits its clinical use. The development of inhibitors with subtype specificity could reduce the problems associated with unselective phosphodiesterase inhibition (321). Macrophages from PDE4B deficient mice indicate that many of the immunomodulatory functions of cAMP appear to be closely linked to this isoform, while knocking out PDE4A or PDE4D showed no such effects (141). A highly selective PDE4B inhibitor has been patented and was being developed for use in lymphoma under the name PLX513, but is currently listed as discontinued by the patent-holder (322). The compound would be an interesting candidate for new treatments not only for sepsis, but also from a range of diseases where unselective PDE4-directed drugs have already demonstrated efficacy; asthma, COPD, psoriasis, rheumatoid arthritis, inflammatory bowel disease, lupus and multiple sclerosis (323-325).

Another promising approach is the possible future use of miRNA-based interventions to achieve targeted immunomodulation in sepsis. An important characteristic of miRNA is that they often control a functionally related cluster of genes, and thus they may serve as switches for larger gene networks aimed at controlling specific biological processes (201;326). In 2008, the first phase I study

of hepatitis C treatment using an antagomiR against the oncogenic mir-122, which also facilitates viral replication, was initiated, and after the trial's successful conclusion, phase II was annouced in 2010 (327). So far, the most common strategy has been to focus on specific miRNA shown to be strongly linked to a single pathogenic process (328). These can be knocked down using either antagomiRs (RNAs conjugated to cholesterol bound to phophorothiotate), "locked nucleic acid" (LNA) constructs, and "miRNA sponges" (competitive inhibitors with multiple binding sites for miRNAs) (329-331).

However, impediments remain before miRNA-based therapies are ready for use in clinical practice. Delivery to the cell has been problematic in vivo, as artificial miRNA probes tend to activate endogenous TLR-receptors, triggering the inflammatory response (326). Also, the nucleic acids are negatively charged and do not easily cross the cell membrane, leading to proposals of encapsulation of the oligonucleotides in liposomes and the use of viral vectors (332). As recently as July 2012, Nature published a study demonstrating how viral delivery of miR-196s was able to ameliorate a serious neurodegenerative and neuromuscular disease in experimental animal models (333).

Our experiments, and the work of others, indicate that targeting miR-142-3p may improve survival in sepsis; blocking the rise in miR-142-3p should blunt the reduction in AC9 mRNA expression, and thus, mitigate the TNFα release from innate immune cells, lessening the chance of the inflammatory process spiraling out of control (237;249). As miRNA probe delivery technology improves, small nucleotide interventions that suppress the miR-142-3p rise in sepsis would be interesting candidates for treatment trials.

In conclusion, the cyclic AMP signaling system is emerging as an important regulator of the innate immune response. Its subcellular structure in cells of the immune system is still poorly understood, and the specific roles of each individual component need to be established. However, a deepened understanding of this complex, but seemingly protective, intracellular signaling system may bring fruitful therapeutic interventions not only to patients suffering from sepsis, but also bring benefits to a wide range of inflammatory diseases.

6. References

(1) Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med 2003 Apr 17;348(16):1546-54.

(2) Flaatten H. Epidemiology of sepsis in Norway in 1999. Crit Care 2004 Aug;8(4):R180-R184.

(3) Tracey KJ. Physiology and immunology of the cholinergic antiinflammatory pathway. J Clin Invest 2007 Feb;117(2):289-96.

(4) Thomas L. The Lives of a Cell: Notes of a Biology Watcher. New York: The Viking Press, Inc.; 1974.

(5) Fry DE. Multiple organ dysfunction syndrome: past, present and future. Surg Infect (Larchmt) 2000;1(3):155-61.

(6) Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Crit Care Med 2008 Jan;36(1):296-327.

(7) Zambon M, Ceola M, Almeida-de-Castro R, Gullo A, Vincent JL. Implementation of the Surviving Sepsis Campaign guidelines for severe sepsis and septic shock: we could go faster. J Crit Care 2008 Dec;23(4):455-60.

(8) Janeway CA, Travers P, Walport M, Slomchick MJ. Immunobiology : the immune system in health and disease. New York: 2001.

(9) Cinel I, Opal SM. Molecular biology of inflammation and sepsis: a primer. Crit Care Med 2009 Jan;37(1):291-304.

(10) Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. J Leukoc Biol 2007 Jan;81(1):1-5.

(11) Strowig T, Henao-Mejia J, Elinav E, Flavell R. Inflammasomes in health and disease. Nature 2012 Jan 19;481(7381):278-86.

(12) Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell 2002 Aug;10(2):417-26.

(13) Shi C, Pamer EG. Monocyte recruitment during infection and inflammation. Nat Rev Immunol 2011 Nov;11(11):762-74.

(14) Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. Nat Rev Immunol 2011 Nov;11(11):723-37.

(15) Bratton DL, Henson PM. Neutrophil clearance: when the party is over, clean-up begins. Trends Immunol 2011 Aug;32(8):350-7.

(16) Gilfillan AM, Beaven MA. Regulation of mast cell responses in health and disease. Crit Rev Immunol 2011;31(6):475-529.

(17) Kita H. Eosinophils: multifaceted biological properties and roles in health and disease. Immunol Rev 2011 Jul;242(1):161-77.

(18) Rothenberg ME, Hogan SP. The eosinophil. Annu Rev Immunol 2006;24:147-74.

(19) Watzl C, Urlaub D. Molecular mechanisms of natural killer cell regulation. Front Biosci 2012;17:1418-32.

(20) Brandstadter JD, Yang Y. Natural killer cell responses to viral infection. J Innate Immun 2011;3(3):274-9.

(21) Iannello A, Debbeche O, Samarani S, Ahmad A. Antiviral NK cell responses in HIV infection: I. NK cell receptor genes as determinants of HIV resistance and progression to AIDS. J Leukoc Biol 2008 Jul;84(1):1-26.

(22) Faveeuw C, Mallevaey T, Trottein F. Role of natural killer T lymphocytes during helminthic infection. Parasite 2008 Sep;15(3):384-8.

(23) Papazahariadou M, Athanasiadis GI, Papadopoulos E, Symeonidou I, Hatzistilianou M, Castellani ML, et al. Involvement of NK cells against tumors and parasites. Int J Biol Markers 2007 Apr;22(2):144-53.

(24) Holtmeier W, Kabelitz D. gammadelta T cells link innate and adaptive immune responses. Chem Immunol Allergy 2005;86:151-83.

(25) Sallusto F, Lanzavecchia A. The instructive role of dendritic cells on T-cell responses. Arthritis Res 2002;4 Suppl 3:S127-S132.

(26) Holst B, Raby AC, Hall JE, Labeta MO. Complement takes its Toll: an inflammatory crosstalk between Toll-like receptors and the receptors for the complement anaphylatoxin C5a. Anaesthesia 2012 Jan;67(1):60-4.

(27) Kak V. Mediators of systemic inflammatory response syndrome and the role of recombinant activated protein C in sepsis syndrome. Infect Dis Clin North Am 2011 Dec;25(4):835-50.

(28) Gay NJ, Keith FJ. Drosophila Toll and IL-1 receptor. Nature 1991 May 30;351(6325):355-6.

(29) Barton GM, Medzhitov R. Toll-like receptor signaling pathways. Science 2003 Jun 6;300(5625):1524-5.

(30) Kim HM, Park BS, Kim JI, Kim SE, Lee J, Oh SC, et al. Crystal structure of the TLR4-MD-2 complex with bound endotoxin antagonist Eritoran. Cell 2007 Sep 7;130(5):906-17.

(31) Jin MS, Kim SE, Heo JY, Lee ME, Kim HM, Paik SG, et al. Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. Cell 2007 Sep 21;130(6):1071-82.

(32) Liu L, Botos I, Wang Y, Leonard JN, Shiloach J, Segal DM, et al. Structural basis of toll-like receptor 3 signaling with double-stranded RNA. Science 2008 Apr 18;320(5874):379-81.

(33) Viriyakosol S, Tobias PS, Kitchens RL, Kirkland TN. MD-2 binds to bacterial lipopolysaccharide. J Biol Chem 2001 Oct 12;276(41):38044-51.

(34) Shimazu R, Akashi S, Ogata H, Nagai Y, Fukudome K, Miyake K, et al. MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. J Exp Med 1999 Jun 7;189(11):1777-82.

(35) Tanamoto K, Azumi S. Salmonella-type heptaacylated lipid A is inactive and acts as an antagonist of lipopolysaccharide action on human line cells. J Immunol 2000 Mar 15;164(6):3149-56.

(36) Jin MS, Lee JO. Structures of the toll-like receptor family and its ligand complexes. Immunity 2008 Aug;29(2):182-91.

(37) Visintin A, Halmen KA, Latz E, Monks BG, Golenbock DT. Pharmacological inhibition of endotoxin responses is achieved by targeting the TLR4 coreceptor, MD-2. J Immunol 2005 Nov 15;175(10):6465-72.

(38) Miyake K. Innate recognition of lipopolysaccharide by CD14 and toll-like receptor 4-MD-2: unique roles for MD-2. Int Immunopharmacol 2003 Jan;3(1):119-28.

(39) Fujihara M, Muroi M, Tanamoto K, Suzuki T, Azuma H, Ikeda H. Molecular mechanisms of macrophage activation and deactivation by lipopolysaccharide: roles of the receptor complex. Pharmacol Ther 2003 Nov;100(2):171-94.

(40) Schumann RR, Leong SR, Flaggs GW, Gray PW, Wright SD, Mathison JC, et al. Structure and function of lipopolysaccharide binding protein. Science 1990 Sep 21;249(4975):1429-31.

(41) Mulero JJ, Boyle BJ, Bradley S, Bright JM, Nelken ST, Ho TT, et al. Three new human members of the lipid transfer/lipopolysaccharide binding protein family (LT/LBP). Immunogenetics 2002 Aug;54(5):293-300.

(42) Ulevitch RJ, Tobias PS. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. Annu Rev Immunol 1995;13:437-57.

(43) Kim JI, Lee CJ, Jin MS, Lee CH, Paik SG, Lee H, et al. Crystal structure of CD14 and its implications for lipopolysaccharide signaling. J Biol Chem 2005 Mar 25;280(12):11347-51.

(44) Sen R, Baltimore D. Inducibility of kappa immunoglobulin enhancer-binding protein Nf-kappa B by a posttranslational mechanism. Cell 1986 Dec 26;47(6):921-8.

(45) Kawai T, Takeuchi O, Fujita T, Inoue J, Muhlradt PF, Sato S, et al. Lipopolysaccharide stimulates the MyD88-independent pathway and results in activation of IFN-regulatory factor 3 and the expression of a subset of lipopolysaccharide-inducible genes. J Immunol 2001 Nov 15;167(10):5887-94.

(46) O'Neill LA. How Toll-like receptors signal: what we know and what we don't know. Curr Opin Immunol 2006 Feb;18(1):3-9.

(47) Bjorkbacka H, Fitzgerald KA, Huet F, Li X, Gregory JA, Lee MA, et al. The induction of macrophage gene expression by LPS predominantly utilizes Myd88-independent signaling cascades. Physiol Genomics 2004 Nov 17;19(3):319-30.

(48) Medzhitov R, Horng T. Transcriptional control of the inflammatory response. Nat Rev Immunol 2009 Oct;9(10):692-703.

(49) Michalek SM, Moore RN, McGhee JR, Rosenstreich DL, Mergenhagen SE. The primary role of lymphoreticular cells in the mediation of host responses to bacterial endotoxim. J Infect Dis 1980 Jan;141(1):55-63.

(50) Guha M, Mackman N. LPS induction of gene expression in human monocytes. Cell Signal 2001 Feb;13(2):85-94.

(51) Michie HR, Manogue KR, Spriggs DR, Revhaug A, O'Dwyer S, Dinarello CA, et al. Detection of circulating tumor necrosis factor after endotoxin administration. N Engl J Med 1988 Jun 9;318(23):1481-6.

(52) Mirlashari MR, Lyberg T. Expression and involvement of Toll-like receptors (TLR)2, TLR4, and CD14 in monocyte TNF-alpha production induced by lipopolysaccharides from Neisseria meningitidis. Med Sci Monit 2003 Aug;9(8):BR316-BR324.

(53) Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, et al. HMG-1 as a late mediator of endotoxin lethality in mice. Science 1999 Jul 9;285(5425):248-51.

(54) Tsujimoto H, Ono S, Hiraki S, Majima T, Kawarabayashi N, Sugasawa H, et al. Hemoperfusion with polymyxin B-immobilized fibers reduced the number of CD16+ CD14+ monocytes in patients with septic shock. J Endotoxin Res 2004;10(4):229-37.

(55) Tsujimoto H, Ono S, Majima T, Kawarabayashi N, Takayama E, Kinoshita M, et al. Neutrophil elastase, MIP-2, and TLR-4 expression during human and experimental sepsis. Shock 2005 Jan;23(1):39-44.

(56) Armstrong L, Medford AR, Hunter KJ, Uppington KM, Millar AB. Differential expression of Toll-like receptor (TLR)-2 and TLR-4 on monocytes in human sepsis. Clin Exp Immunol 2004 May;136(2):312-9.

(57) Williams DL, Ha T, Li C, Kalbfleisch JH, Schweitzer J, Vogt W, et al. Modulation of tissue Toll-like receptor 2 and 4 during the early phases of polymicrobial sepsis correlates with mortality. Crit Care Med 2003 Jun;31(6):1808-18.

(58) Paterson HM, Murphy TJ, Purcell EJ, Shelley O, Kriynovich SJ, Lien E, et al. Injury primes the innate immune system for enhanced Toll-like receptor reactivity. J Immunol 2003 Aug 1;171(3):1473-83.

(59) Tsujimoto H, Ono S, Efron PA, Scumpia PO, Moldawer LL, Mochizuki H. Role of Toll-like receptors in the development of sepsis. Shock 2008 Mar;29(3):315-21.

(60) Wang YY, Dahle MK, Agren J, Myhre AE, Reinholt FP, Foster SJ, et al. Activation of the liver X receptor protects against hepatic injury in endotoxemia by suppressing Kupffer cell activation. Shock 2006 Feb;25(2):141-6.

(61) ACCP/CCM. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med 1992 Jun;20(6):864-74.

(62) Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Intensive Care Med 2003 Apr;29(4):530-8.

(63) Pearson RD, Symes P, Conboy M, Weiss AA, Hewlett EL. Inhibition of monocyte oxidative responses by Bordetella pertussis adenylate cyclase toxin. J Immunol 1987 Oct 15;139(8):2749-54.

(64) Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003 Jan 9;348(2):138-50.

(65) Wang TS, Deng JC. Molecular and cellular aspects of sepsis-induced immunosuppression. J Mol Med 2008 May;86(5):495-506.

(66) Angele MK, Faist E. Clinical review: immunodepression in the surgical patient and increased susceptibility to infection. Crit Care 2002 Aug;6(4):298-305.

(67) Osuchowski MF, Welch K, Siddiqui J, Remick DG. Circulating cytokine/inhibitor profiles reshape the understanding of the SIRS/CARS continuum in sepsis and predict mortality. J Immunol 2006 Aug 1;177(3):1967-74.

(68) Osuchowski MF, Welch K, Yang H, Siddiqui J, Remick DG. Chronic sepsis mortality characterized by an individualized inflammatory response. J Immunol 2007 Jul 1;179(1):623-30.

(69) Xiao H, Siddiqui J, Remick DG. Mechanisms of mortality in early and late sepsis. Infect Immun 2006 Sep;74(9):5227-35.

(70) Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001 Jul;29(7):1303-10.

(71) Wichmann MW, Inthorn D, Andress HJ, Schildberg FW. Incidence and mortality of severe sepsis in surgical intensive care patients: the influence of patient gender on disease process and outcome. Intensive Care Med 2000 Feb;26(2):167-72.

(72) Adrie C, Azoulay E, Francais A, Clec'h C, Darques L, Schwebel C, et al. Influence of gender on the outcome of severe sepsis: a reappraisal. Chest 2007 Dec;132(6):1786-93.

(73) Berkowitz DM, Martin GS. Sepsis and sex: can we look beyond our hormones? Chest 2007 Dec;132(6):1725-7.

(74) Wafaisade A, Lefering R, Bouillon B, Sakka SG, Thamm OC, Paffrath T, et al. Epidemiology and risk factors of sepsis after multiple trauma: an analysis of 29,829 patients from the Trauma Registry of the German Society for Trauma Surgery. Crit Care Med 2011 Apr;39(4):621-8.

(75) Singh NK, Singh VK, Guleria R. Potential confounders in the "Influence of gender on the outcome of severe sepsis" study. Chest 2008 Sep;134(3):671-2.

(76) Mahmood K, Eldeirawi K, Wahidi MM. Association of gender with outcomes in critically ill patients. Crit Care 2012 May 22;16(3):R92.

(77) Kawasaki T, Chaudry IH. The effects of estrogen on various organs: therapeutic approach for sepsis, trauma, and reperfusion injury. Part 2: liver, intestine, spleen, and kidney. J Anesth 2012 Jun 23.

(78) Pietropaoli AP, Glance LG, Oakes D, Fisher SG. Gender differences in mortality in patients with severe sepsis or septic shock. Gend Med 2010 Oct;7(5):422-37.

(79) George RL, McGwin G, Jr., Metzger J, Chaudry IH, Rue LW, III. The association between gender and mortality among trauma patients as modified by age. J Trauma 2003 Mar;54(3):464-71.

(80) Nachtigall I, Tafelski S, Rothbart A, Kaufner L, Schmidt M, Tamarkin A, et al. Gender-related outcome difference is related to course of sepsis on mixed ICUs: a prospective, observational clinical study. Crit Care 2011;15(3):R151.

(81) Sperry JL, Nathens AB, Frankel HL, Vanek SL, Moore EE, Maier RV, et al. Characterization of the gender dimorphism after injury and hemorrhagic shock: are hormonal differences responsible? Crit Care Med 2008 Jun;36(6):1838-45.

(82) Safdar B, McGregor AJ, McKee SA, Ali A, Radulescu R, Himelfarb NT, et al. Inclusion of gender in emergency medicine research. Acad Emerg Med 2011 Feb;18(2):e1-e4.

(83) van Eijk LT, Dorresteijn MJ, Smits P, van der Hoeven JG, Netea MG, Pickkers P. Gender differences in the innate immune response and vascular reactivity following the administration of endotoxin to human volunteers. Crit Care Med 2007 Jun;35(6):1464-9.

(84) Coyle SM, Calvano SE, Lowry SF. Gender influences in vivo human responses to endotoxin. Shock 2006 Dec;26(6):538-43.

(85) Marriott I, Huet-Hudson YM. Sexual dimorphism in innate immune responses to infectious organisms. Immunol Res 2006;34(3):177-92.

(86) Moxley G, Posthuma D, Carlson P, Estrada E, Han J, Benson LL, et al. Sexual dimorphism in innate immunity. Arthritis Rheum 2002 Jan;46(1):250-8.

(87) Suffredini AF. Systemic inflammation and sexual dimorphism: more than meets the eye. Crit Care Med 2007 Jun;35(6):1610-2.

(88) Timmons BW, Hamadeh MJ, Devries MC, Tarnopolsky MA. Influence of gender, menstrual phase, and oral contraceptive use on immunological changes in response to prolonged cycling. J Appl Physiol 2005 Sep;99(3):979-85.

(89) Combes A, Luyt CE, Trouillet JL, Nieszkowska A, Chastre J. Gender impact on the outcomes of critically ill patients with nosocomial infections. Crit Care Med 2009 Sep;37(9):2506-11.

(90) Serezani CH, Ballinger MN, Aronoff DM, Peters-Golden M. Cyclic AMP: master regulator of innate immune cell function. Am J Respir Cell Mol Biol 2008 Aug;39(2):127-32.

(91) Tasken K, Aandahl EM. Localized effects of cAMP mediated by distinct routes of protein kinase A. Physiol Rev 2004 Jan;84(1):137-67.

(92) Luttrell LM. Transmembrane signaling by G protein-coupled receptors. Methods Mol Biol 2006;332:3-49.

(93) Landry Y, Niederhoffer N, Sick E, Gies JP. Heptahelical and other G-protein-coupled receptors (GPCRs) signaling. Curr Med Chem 2006;13(1):51-63.

(94) Calebiro D, Nikolaev VO, Persani L, Lohse MJ. Signaling by internalized G-protein-coupled receptors. Trends Pharmacol Sci 2010 May;31(5):221-8.

(95) Hanoune J, Defer N. Regulation and role of adenylyl cyclase isoforms. Annu Rev Pharmacol Toxicol 2001;41:145-74.

(96) Zaccolo M. cAMP signal transduction in the heart: understanding spatial control for the development of novel therapeutic strategies. Br J Pharmacol 2009 Sep;158(1):50-60.

(97) Mongillo M, Terrin A, Evellin S, Lissandron V, Zaccolo M. Study of cyclic adenosine monophosphate microdomains in cells. Methods Mol Biol 2005;307:1-13.

(98) Willoughby D, Cooper DM. Organization and Ca2+ regulation of adenylyl cyclases in cAMP microdomains. Physiol Rev 2007 Jul;87(3):965-1010.

(99) Willoughby D, Cooper DM. Live-cell imaging of cAMP dynamics. Nat Methods 2008 Jan;5(1):29-36.

(100) Meyer G, Guizzardi F, Rodighiero S, Manfredi R, Saino S, Sironi C, et al. Ion transport across the gallbladder epithelium. Curr Drug Targets Immune Endocr Metabol Disord 2005 Jun;5(2):143-51.

(101) Chambers LA, Rollins BM, Tarran R. Liquid movement across the surface epithelium of large airways. Respir Physiol Neurobiol 2007 Dec 15;159(3):256-70.

(102) Holz GG, Kang G, Harbeck M, Roe MW, Chepurny OG. Cell physiology of cAMP sensor Epac. J Physiol 2006 Nov 15;577(Pt 1):5-15.

(103) Dodge-Kafka KL, Kapiloff MS. The mAKAP signaling complex: integration of cAMP, calcium, and MAP kinase signaling pathways. Eur J Cell Biol 2006 Jul;85(7):593-602.

(104) Kopperud R, Krakstad C, Selheim F, Doskeland SO. cAMP effector mechanisms. Novel twists for an 'old' signaling system. FEBS Lett 2003 Jul 3;546(1):121-6.

(105) Dessauer CW. Adenylyl cyclase--A-kinase anchoring protein complexes: the next dimension in cAMP signaling. Mol Pharmacol 2009 Nov;76(5):935-41.

(106) Enserink JM, Christensen AE, de RJ, van TM, Schwede F, Genieser HG, et al. A novel Epac-specific cAMP analogue demonstrates independent regulation of Rap1 and ERK. Nat Cell Biol 2002 Nov;4(11):901-6.

(107) Borland G, Smith BO, Yarwood SJ. EPAC proteins transduce diverse cellular actions of cAMP. Br J Pharmacol 2009 Feb 6.

(108) Dahle MK, Risoe PK, Wang JE, Aasen AO. Cyclic adenosine monophosphate signaling and organ dysfunction in septic shock. Journal of Organ Dysfunction 2008 Nov 22;5(1):38-50.

(109) Bryn T, Mahic M, Aandahl EM, Froland SS, Aukrust P, Tasken K. Inhibition of protein kinase A improves effector function of monocytes from HIV-infected patients. AIDS Res Hum Retroviruses 2008 Jul;24(7):1013-5.

(110) Houslay MD, Schafer P, Zhang KY. Keynote review: phosphodiesterase-4 as a therapeutic target. Drug Discov Today 2005 Nov 15;10(22):1503-19.

(111) Hatzimouratidis K, Hatzichristou DG. Looking to the future for erectile dysfunction therapies. Drugs 2008;68(2):231-50.

(112) Scott JD. Compartmentalized cAMP signalling: a personal perspective. Biochem Soc Trans 2006 Aug;34(Pt 4):465-7.

(113) Jarnaess E, Tasken K. Spatiotemporal control of cAMP signalling processes by anchored signalling complexes. Biochem Soc Trans 2007 Nov;35(Pt 5):931-7.

(114) Lorenowicz MJ, Fernandez-Borja M, Hordijk PL. cAMP signaling in leukocyte transendothelial migration. Arterioscler Thromb Vasc Biol 2007 May;27(5):1014-22.

(115) Insel PA, Head BP, Patel HH, Roth DM, Bundey RA, Swaney JS. Compartmentation of G-protein-coupled receptors and their signalling components in lipid rafts and caveolae. Biochem Soc Trans 2005 Nov;33(Pt 5):1131-4.

(116) Gold MG, Lygren B, Dokurno P, Hoshi N, McConnachie G, Tasken K, et al. Molecular basis of AKAP specificity for PKA regulatory subunits. Mol Cell 2006 Nov 3;24(3):383-95.

(117) Ostrom RS, Bundey RA, Insel PA. Nitric oxide inhibition of adenylyl cyclase type 6 activity is dependent upon lipid rafts and caveolin signaling complexes. J Biol Chem 2004 May 7;279(19):19846-53.

(118) Wen AY, Sakamoto KM, Miller LS. The role of the transcription factor CREB in immune function. J Immunol 2010 Dec 1;185(11):6413-9.

(119) de RJ, Zwartkruis FJ, Verheijen MH, Cool RH, Nijman SM, Wittinghofer A, et al. Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. Nature 1998 Dec 3;396(6710):474-7.

(120) Kawasaki H, Springett GM, Mochizuki N, Toki S, Nakaya M, Matsuda M, et al. A family of cAMP-binding proteins that directly activate Rap1. Science 1998 Dec 18;282(5397):2275-9.

(121) Bos JL. Linking Rap to cell adhesion. Curr Opin Cell Biol 2005 Apr;17(2):123-8.

(122) Cullere X, Shaw SK, Andersson L, Hirahashi J, Luscinskas FW, Mayadas TN. Regulation of vascular endothelial barrier function by Epac, a cAMP-activated exchange factor for Rap GTPase. Blood 2005 Mar 1;105(5):1950-5.

(123) Fukuhara S, Sakurai A, Sano H, Yamagishi A, Somekawa S, Takakura N, et al. Cyclic AMP potentiates vascular endothelial cadherin-mediated cell-cell contact to enhance endothelial barrier function through an Epac-Rap1 signaling pathway. Mol Cell Biol 2005 Jan;25(1):136-46.

(124) Lorenowicz MJ, van GJ, de BM, Hordijk PL, Fernandez-Borja M. Epac1-Rap1 signaling regulates monocyte adhesion and chemotaxis. J Leukoc Biol 2006 Dec;80(6):1542-52.

(125) Rangarajan S, Enserink JM, Kuiperij HB, de RJ, Price LS, Schwede F, et al. Cyclic AMP induces integrin-mediated cell adhesion through Epac and Rap1 upon stimulation of the beta 2-adrenergic receptor. J Cell Biol 2003 Feb 17;160(4):487-93.

(126) Wittchen ES, van Buul JD, Burridge K, Worthylake RA. Trading spaces: Rap, Rac, and Rho as architects of transendothelial migration. Curr Opin Hematol 2005 Jan;12(1):14-21.

(127) Houslay MD. Underpinning compartmentalised cAMP signalling through targeted cAMP breakdown. Trends Biochem Sci 2010 Feb;35(2):91-100.

(128) Breckler M, Berthouze M, Laurent AC, Crozatier B, Morel E, Lezoualc'h F. Rap-linked cAMP signaling Epac proteins: compartmentation, functioning and disease implications. Cell Signal 2011 Aug;23(8):1257-66.

(129) Cheng X, Ji Z, Tsalkova T, Mei F. Epac and PKA: a tale of two intracellular cAMP receptors. Acta Biochim Biophys Sin (Shanghai) 2008 Jul;40(7):651-62.

(130) Lynch MJ, Hill EV, Houslay MD. Intracellular targeting of phosphodiesterase-4 underpins compartmentalized cAMP signaling. Curr Top Dev Biol 2006;75:225-59.

(131) Xiang YK. Compartmentalization of beta-adrenergic signals in cardiomyocytes. Circ Res 2011 Jul 8;109(2):231-44.

(132) Aronoff DM, Carstens JK, Chen GH, Toews GB, Peters-Golden M. Short communication: differences between macrophages and dendritic cells in the cyclic AMP-dependent regulation of lipopolysaccharide-induced cytokine and chemokine synthesis. J Interferon Cytokine Res 2006 Nov;26(11):827-33.

(133) Bryn T, Mahic M, Enserink JM, Schwede F, Aandahl EM, Tasken K. The cyclic AMP-Epac1-Rap1 pathway is dissociated from regulation of effector functions in monocytes but acquires immunoregulatory function in mature macrophages. J Immunol 2006 Jun 15;176(12):7361-70.

(134) Dahle MK, Myhre AE, Aasen AO, Wang JE. Effects of forskolin on Kupffer cell production of interleukin-10 and tumor necrosis factor alpha differ from those of endogenous adenylyl cyclase activators: possible role for adenylyl cyclase 9. Infect Immun 2005 Nov;73(11):7290-6.

(135) Gasperini S, Crepaldi L, Calzetti F, Gatto L, Berlato C, Bazzoni F, et al. Interleukin-10 and cAMP-elevating agents cooperate to induce suppressor of cytokine signaling-3 via a protein kinase A-independent signal. Eur Cytokine Netw 2002 Jan;13(1):47-53.

(136) van der Pouw Kraan TC, Boeije LC, Smeenk RJ, Wijdenes J, Aarden LA. Prostaglandin-E2 is a potent inhibitor of human interleukin 12 production. J Exp Med 1995 Feb 1;181(2):775-9.

(137) van der Pouw Kraan TC, Boeije LC, Snijders A, Smeenk RJ, Wijdenes J, Aarden LA. Regulation of IL-12 production by human monocytes and the influence of prostaglandin E2. Ann N Y Acad Sci 1996 Oct 31;795:147-57.

(138) Harzenetter MD, Novotny AR, Gais P, Molina CA, Altmayr F, Holzmann B. Negative regulation of TLR responses by the neuropeptide CGRP is mediated by the transcriptional repressor ICER. J Immunol 2007 Jul 1;179(1):607-15.

(139) Ollivier V, Parry GC, Cobb RR, de PD, Mackman N. Elevated cyclic AMP inhibits NF-kappaB-mediated transcription in human monocytic cells and endothelial cells. J Biol Chem 1996 Aug 23;271(34):20828-35.

(140) Parry GC, Mackman N. Role of cyclic AMP response element-binding protein in cyclic AMP inhibition of NF-kappaB-mediated transcription. J Immunol 1997 Dec 1;159(11):5450-6.

(141) Jin SL, Lan L, Zoudilova M, Conti M. Specific role of phosphodiesterase 4B in lipopolysaccharide-induced signaling in mouse macrophages. J Immunol 2005 Aug 1;175(3):1523-31.

(142) Brown DM, Hutchison L, Donaldson K, MacKenzie SJ, Dick CA, Stone V. The effect of oxidative stress on macrophages and lung epithelial cells: the role of phosphodiesterases 1 and 4. Toxicol Lett 2007 Jan 10;168(1):1-6.

(143) Latifi SQ, O'Riordan MA, Levine AD. Interleukin-10 controls the onset of irreversible septic shock. Infect Immun 2002 Aug;70(8):4441-6.

(144) Ananieva O, Darragh J, Johansen C, Carr JM, McIlrath J, Park JM, et al. The kinases MSK1 and MSK2 act as negative regulators of Toll-like receptor signaling. Nat Immunol 2008 Sep;9(9):1028-36.

(145) Xu XJ, Reichner JS, Mastrofrancesco B, Henry WL, Jr., Albina JE. Prostaglandin E2 suppresses lipopolysaccharide-stimulated IFN-beta production. J Immunol 2008 Feb 15;180(4):2125-31.

(146) Hareng L, Meergans T, von AS, Volk HD, Hartung T. Cyclic AMP increases endogenous granulocyte colony-stimulating factor formation in monocytes and THP-1 macrophages despite attenuated TNF-alpha formation. Eur J Immunol 2003 Aug;33(8):2287-96.

(147) Brenner S, Prosch S, Schenke-Layland K, Riese U, Gausmann U, Platzer C. cAMP-induced Interleukin-10 promoter activation depends on CCAAT/enhancer-binding protein expression and monocytic differentiation. J Biol Chem 2003 Feb 21;278(8):5597-604.

(148) Grandoch M, Roscioni SS, Schmidt M. The role of Epac proteins, novel cAMP mediators, in the regulation of immune, lung and neuronal function. Br J Pharmacol 2010 Jan 1;159(2):265-84.

(149) Nambu M, Morita M, Watanabe H, Uenoyama Y, Kim KM, Tanaka M, et al. Regulation of Fc gamma receptor expression and phagocytosis of a human monoblast cell line U937. Participation of cAMP and protein kinase C in the effects of IFN-gamma and phorbol ester. J Immunol 1989 Dec 15;143(12):4158-65.

(150) Atkinson JP, Michael JM, Chaplin H, Jr., Parker CW. Modulation of macrophage C3b receptor function by cytochalasin-sensitive structures. J Immunol 1977 Apr;118(4):1292-9.

(151) Makranz C, Cohen G, Reichert F, Kodama T, Rotshenker S. cAMP cascade (PKA, Epac, adenylyl cyclase, Gi, and phosphodiesterases) regulates myelin phagocytosis mediated by complement receptor-3 and scavenger receptor-AI/II in microglia and macrophages. Glia 2006 Mar;53(4):441-8.

(152) Ydrenius L, Majeed M, Rasmusson BJ, Stendahl O, Sarndahl E. Activation of cAMP-dependent protein kinase is necessary for actin rearrangements in human neutrophils during phagocytosis. J Leukoc Biol 2000 Apr;67(4):520-8.

(153) Zalavary S, Bengtsson T. Adenosine inhibits actin dynamics in human neutrophils: evidence for the involvement of cAMP. Eur J Cell Biol 1998 Feb;75(2):128-39.

(154) Hazan-Eitan Z, Weinstein Y, Hadad N, Konforty A, Levy R. Induction of Fc gammaRIIA expression in myeloid PLB cells during differentiation depends on cytosolic phospholipase A2 activity and is regulated via activation of CREB by PGE2. Blood 2006 Sep 1;108(5):1758-66.

(155) Aronoff DM, Canetti C, Serezani CH, Luo M, Peters-Golden M. Cutting edge: macrophage inhibition by cyclic AMP (cAMP): differential roles of protein kinase A and exchange protein directly activated by cAMP-1. J Immunol 2005 Jan 15;174(2):595-9.

(156) Kalamidas SA, Kuehnel MP, Peyron P, Rybin V, Rauch S, Kotoulas OB, et al. cAMP synthesis and degradation by phagosomes regulate actin assembly and fusion events: consequences for mycobacteria. J Cell Sci 2006 Sep 1;119(Pt 17):3686-94.

(157) Di A, Brown ME, Deriy LV, Li C, Szeto FL, Chen Y, et al. CFTR regulates phagosome acidification in macrophages and alters bactericidal activity. Nat Cell Biol 2006 Sep;8(9):933-44.

(158) Pryzwansky KB, Kidao S, Merricks EP. Compartmentalization of PDE-4 and cAMP-dependent protein kinase in neutrophils and macrophages during phagocytosis. Cell Biochem Biophys 1998;28(2-3):251-75.

(159) Brock TG, Serezani CH, Carstens JK, Peters-Golden M, Aronoff DM. Effects of prostaglandin E2 on the subcellular localization of Epac-1 and Rap1 proteins during Fcgamma-receptor-mediated phagocytosis in alveolar macrophages. Exp Cell Res 2008 Jan 15;314(2):255-63.

(160) O'Dorisio MS, Vandenbark GR, LoBuglio AF. Human monocyte killing of Staphylococcus aureus: modulation by agonists of cyclic adenosine 3',5'-monophosphate and cyclic guanosine 3',5'-monophosphate. Infect Immun 1979 Nov;26(2):604-10.

(161) Serezani CH, Chung J, Ballinger MN, Moore BB, Aronoff DM, Peters-Golden M. Prostaglandin E2 suppresses bacterial killing in alveolar macrophages by inhibiting NADPH oxidase. Am J Respir Cell Mol Biol 2007 Nov;37(5):562-70.

(162) Nokta MA, Pollard RB. Human immunodeficiency virus replication: modulation by cellular levels of cAMP. AIDS Res Hum Retroviruses 1992 Jul;8(7):1255-61.

(163) Fulop T, Jr., Foris G, Worum I, Leovey A. Age-dependent alterations of Fc gamma receptor-mediated effector functions of human polymorphonuclear leucocytes. Clin Exp Immunol 1985 Aug;61(2):425-32.

(164) Wirth JJ, Kierszenbaum F. Macrophage mediation of the inhibitory effects of elevated intracellular levels of adenosine-3':5' cyclic monophosphate (cAMP) on macrophage-Trypanosoma cruzi association. Int J Parasitol 1984 Aug;14(4):401-4.

(165) Ariga M, Neitzert B, Nakae S, Mottin G, Bertrand C, Pruniaux MP, et al. Nonredundant function of phosphodiesterases 4D and 4B in neutrophil recruitment to the site of inflammation. J Immunol 2004 Dec 15;173(12):7531-8.

(166) Lin P, Welch EJ, Gao XP, Malik AB, Ye RD. Lysophosphatidylcholine modulates neutrophil oxidant production through elevation of cyclic AMP. J Immunol 2005 Mar 1;174(5):2981-9.

(167) Bengis-Garber C, Gruener N. Protein kinase A downregulates the phosphorylation of p47 phox in human neutrophils: a possible pathway for inhibition of the respiratory burst. Cell Signal 1996 Jun;8(4):291-6.

(168) Chang YC, Li PC, Chen BC, Chang MS, Wang JL, Chiu WT, et al. Lipoteichoic acid-induced nitric oxide synthase expression in RAW 264.7 macrophages is mediated by cyclooxygenase-2, prostaglandin E2, protein kinase A, p38 MAPK, and nuclear factor-kappaB pathways. Cell Signal 2006 Aug;18(8):1235-43.

(169) Chen CC, Chiu KT, Sun YT, Chen WC. Role of the cyclic AMP-protein kinase A pathway in lipopolysaccharide-induced nitric oxide synthase expression in RAW 264.7 macrophages. Involvement of cyclooxygenase-2. J Biol Chem 1999 Oct 29;274(44):31559-64.

(170) Won JS, Im YB, Singh AK, Singh I. Dual role of cAMP in iNOS expression in glial cells and macrophages is mediated by differential regulation of p38-MAPK/ATF-2 activation and iNOS stability. Free Radic Biol Med 2004 Dec 1;37(11):1834-44.

(171) Mustafa SB, Olson MS. Expression of nitric-oxide synthase in rat Kupffer cells is regulated by cAMP. J Biol Chem 1998 Feb 27;273(9):5073-80.

(172) Basoni C, Nobles M, Grimshaw A, Desgranges C, Davies D, Perretti M, et al. Inhibitory control of TGF-beta1 on the activation of Rap1, CD11b, and transendothelial migration of leukocytes. FASEB J 2005 May;19(7):822-4.

(173) Berends C, Dijkhuizen B, de Monchy JG, Dubois AE, Gerritsen J, Kauffman HF. Inhibition of PAF-induced expression of CD11b and shedding of L-selectin on human neutrophils and eosinophils by the type IV selective PDE inhibitor, rolipram. Eur Respir J 1997 May;10(5):1000-7.

(174) Jones SL. Protein kinase A regulates beta2 integrin avidity in neutrophils. J Leukoc Biol 2002 Jun;71(6):1042-8.

(175) Curnow RT, Rayfield EJ, George DT, Zenser TV, DeRubertis FR. Altered hepatic glycogen metabolism and glucoregulatory hormones during sepsis. Am J Physiol 1976 May;230(5):1296-301.

(176) Zenser TV, DeRubertis FR, George DT, Rayfield EJ. Infection-induced hyperglucagonemia and altered hepatic response to glucagon in the rat. Am J Physiol 1974 Dec;227(6):1299-305.

(177) Ghosh S, Liu MS. Decrease in adenylate cyclase activity in dog livers during endotoxic shock. Am J Physiol 1983 Nov;245(5 Pt 1):R737-R742.

(178) Goto F, Fujita T, Otani E, Yamamuro M. The effect of indomethacin and adrenergic receptor blocking agents on rats and canine responses to endotoxin. Circ Shock 1980;7(4):413-24.

(179) Romano FD, Jones SB. Beta-adrenergic stimulation of myocardial cyclic AMP in endotoxic rats. Circ Shock 1985;17(3):243-52.

(180) Bernardin G, Strosberg AD, Bernard A, Mattei M, Marullo S. Beta-adrenergic receptor-dependent and -independent stimulation of adenylate cyclase is impaired during severe sepsis in humans. Intensive Care Med 1998 Dec;24(12):1315-22.

(181) Bernardin G, Kisoka RL, Delporte C, Robberecht P, Vincent JL. Impairment of beta-adrenergic signaling in healthy peripheral blood mononuclear cells exposed to serum from patients with septic shock: involvement of the inhibitory pathway of adenylyl cyclase stimulation. Shock 2003 Feb;19(2):108-12.

(182) Delgado M, Leceta J, Ganea D. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit the production of inflammatory mediators by activated microglia. J Leukoc Biol 2003 Jan;73(1):155-64.

(183) Sullivan GW, Linden J. Role of A(2A) adenosine receptors in inflammation. Drug Development Research 1998;45(3-4):103-12.

(184) Ahuja N, Kumar P, Bhatnagar R. The adenylate cyclase toxins. Crit Rev Microbiol 2004;30(3):187-96.

(185) Vanden Broeck D, Horvath C, De Wolf MJ. Vibrio cholerae: cholera toxin. Int J Biochem Cell Biol 2007;39(10):1771-5.

(186) Niemialtowski M, Klucinski W, Malicki K, de Faundez IS. Cholera toxin (choleragen)-polymorphonuclear leukocyte interactions: effect on migration in vitro and Fc gamma R-dependent phagocytic and bactericidal activity. Microbiol Immunol 1993;37(1):55-62.

(187) Braun MC, He J, Wu CY, Kelsall BL. Cholera toxin suppresses interleukin (IL)-12 production and IL-12 receptor beta1 and beta2 chain expression. J Exp Med 1999 Feb 1;189(3):541-52.

(188) Bergman MJ, Guerrant RL, Murad F, Richardson SH, Weaver D, Mandell GL. Interaction of polymorphonuclear neutrophils with Escherichia coli. Effect of enterotoxin on phagocytosis, killing, chemotaxis, and cyclic AMP. J Clin Invest 1978 Feb;61(2):227-34.

(189) Vojtova J, Kamanova J, Sebo P. Bordetella adenylate cyclase toxin: a swift saboteur of host defense. Curr Opin Microbiol 2006 Feb;9(1):69-75.

(190) Katada T, Ui M. Direct modification of the membrane adenylate cyclase system by islet-activating protein due to ADP-ribosylation of a membrane protein. Proc Natl Acad Sci U S A 1982 May;79(10):3129-33.

(191) Hiemstra PS, Annema A, Schippers EF, van FR. Pertussis toxin partially inhibits phagocytosis of immunoglobulin G-opsonized Staphylococcus aureus by human granulocytes but does not affect intracellular killing. Infect Immun 1992 Jan;60(1):202-5.

(192) Schaeffer LM, Weiss AA. Pertussis toxin and lipopolysaccharide influence phagocytosis of Bordetella pertussis by human monocytes. Infect Immun 2001 Dec;69(12):7635-41.

(193) Kirimanjeswara GS, Agosto LM, Kennett MJ, Bjornstad ON, Harvill ET. Pertussis toxin inhibits neutrophil recruitment to delay antibody-mediated clearance of Bordetella pertussis. J Clin Invest 2005 Dec;115(12):3594-601.

(194) Confer DL, Eaton JW. Phagocyte impotence caused by an invasive bacterial adenylate cyclase. Science 1982 Sep 3;217(4563):948-50.

(195) Sonkoly E, Pivarcsi A. Advances in microRNAs: implications for immunity and inflammatory diseases. J Cell Mol Med 2009 Jan;13(1):24-38.

(196) Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993 Dec 3;75(5):843-54.

(197) Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 2009 Jan;19(1):92-105.

(198) Sonkoly E, Stahle M, Pivarcsi A. MicroRNAs and immunity: novel players in the regulation of normal immune function and inflammation. Semin Cancer Biol 2008 Apr;18(2):131-40.

(199) Tili E, Michaille JJ, Costinean S, Croce CM. MicroRNAs, the immune system and rheumatic disease. Nat Clin Pract Rheumatol 2008 Oct;4(10):534-41.

(200) Tiscornia G, Izpisua Belmonte JC. MicroRNAs in embryonic stem cell function and fate. Genes Dev 2010 Dec 15;24(24):2732-41.

(201) John B, Enright AJ, Aravin A, Tuschl T, Sander C, Marks DS. Human MicroRNA targets. PLoS Biol 2004 Nov;2(11):e363.

(202) Duroux-Richard I, Presumey J, Courties G, Gay S, Gordeladze J, Jorgensen C, et al. MicroRNAs as new player in rheumatoid arthritis. Joint Bone Spine 2011 Jan;78(1):17-22.

(203) Zomer A, Vendrig T, Hopmans ES, van EM, Middeldorp JM, Pegtel DM. Exosomes: Fit to deliver small RNA. Commun Integr Biol 2010 Sep;3(5):447-50.

(204) Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci U S A 2006 Aug 15;103(33):12481-6.

(205) Nahid MA, Pauley KM, Satoh M, Chan EK. miR-146a is critical for endotoxin-induced tolerance: IMPLICATION IN INNATE IMMUNITY. J Biol Chem 2009 Dec 11;284(50):34590-9.

(206) Nahid MA, Satoh M, Chan EK. Mechanistic role of microRNA-146a in endotoxin-induced differential cross-regulation of TLR signaling. J Immunol 2011 Feb 1;186(3):1723-34.

(207) Tili E, Michaille JJ, Cimino A, Costinean S, Dumitru CD, Adair B, et al. Modulation of miR-155 and miR-125b levels following lipopolysaccharide/TNF-alpha stimulation and their possible roles in regulating the response to endotoxin shock. J Immunol 2007 Oct 15;179(8):5082-9.

(208) Fassi FL, Koch M, Belogolova E, Khalil H, Bolz C, Kalali B, et al. Helicobacter pylori induces miR-155 in T cells in a cAMP-Foxp3-dependent manner. PLoS One 2010;5(3):e9500.

(209) Huang B, Zhao J, Lei Z, Shen S, Li D, Shen GX, et al. miR-142-3p restricts cAMP production in CD4+. EMBO Rep 2009 Feb;10(2):180-5.

(210) Murphy AJ, Guyre PM, Pioli PA. Estradiol suppresses NF-kappa B activation through coordinated regulation of let-7a and miR-125b in primary human macrophages. J Immunol 2010 May 1;184(9):5029-37.

(211) Moschos SA, Williams AE, Perry MM, Birrell MA, Belvisi MG, Lindsay MA. Expression profiling in vivo demonstrates rapid changes in lung microRNA levels following lipopolysaccharide-induced inflammation but not in the anti-inflammatory action of glucocorticoids. BMC Genomics 2007;8:240.

(212) Niu Y, Mo D, Qin L, Wang C, Li A, Zhao X, et al. Lipopolysaccharide-induced miR-1224 negatively regulates tumour necrosis factor-alpha gene expression by modulating Sp1. Immunology 2011 May;133(1):8-20.

(213) Liu C, Gao F, Li B, Mitchel RE, Liu X, Lin J, et al. TLR4 knockout protects mice from radiation-induced thymic lymphoma by downregulation of IL6 and miR-21. Leukemia 2011 Sep;25(9):1516-9.

(214) Wang JF, Yu ML, Yu G, Bian JJ, Deng XM, Wan XJ, et al. Serum miR-146a and miR-223 as potential new biomarkers for sepsis. Biochem Biophys Res Commun 2010 Mar 26;394(1):184-8.

(215) Risoe PK, Wang Y, Stuestol JF, Aasen AO, Wang JE, Dahle MK. Lipopolysaccharide attenuates mRNA levels of several adenylyl cyclase isoforms in vivo. Biochim Biophys Acta 2007 Jan;1772(1):32-9.

(216) Wang YY, Dahle MK, Steffensen KR, Reinholt FP, Collins JL, Thiemermann C, et al. Liver X receptor agonist GW3965 dose-dependently regulates lps-mediated liver injury and modulates posttranscriptional TNF-alpha production and p38 mitogen-activated protein kinase activation in liver macrophages. Shock 2009 Nov;32(5):548-53.

(217) Hubbard WJ, Choudhry M, Schwacha MG, Kerby JD, Rue LW, III, Bland KI, et al. Cecal ligation and puncture. Shock 2005 Dec;24 Suppl 1:52-7.

(218) Maddens B, Vandendriessche B, Demon D, Vanholder R, Chiers K, Cauwels A, et al. Severity of sepsis-induced acute kidney injury in a novel mouse model is age dependent. Crit Care Med 2012 Jun 28.

(219) Leelahavanichkul A, Bocharov AV, Kurlander R, Baranova IN, Vishnyakova TG, Souza AC, et al. Class B scavenger receptor types I and II and CD36 targeting improves sepsis survival and acute outcomes in mice. J Immunol 2012 Mar 15;188(6):2749-58.

(220) Wang JE, Solberg R, Okkenhaug C, Jorgensen PF, Krohn CD, Aasen AO. Cytokine modulation in experimental endotoxemia: characterization of an ex vivo whole blood model. Eur Surg Res 2000;32(2):65-73.

(221) Peri F, Piazza M. Therapeutic targeting of innate immunity with Toll-like receptor 4 (TLR4) antagonists. Biotechnol Adv 2012 Jan;30(1):251-60.

(222) Wang JE, Jorgensen PF, Almlof M, Thiemermann C, Foster SJ, Aasen AO, et al. Peptidoglycan and lipoteichoic acid from Staphylococcus aureus induce tumor necrosis factor alpha, interleukin 6 (IL-6), and IL-10 production in both T cells and monocytes in a human whole blood model. Infect Immun 2000 Jul;68(7):3965-70.

(223) Knook DL, Brouwer A. Kupffer cells and the acute phase response: the effect of aging. Immunol Invest 1989 Jan;18(1-4):339-50.

(224) Knook DL, Blansjaar N, Sleyster EC. Isolation and characterization of Kupffer and endothelial cells from the rat liver. Exp Cell Res 1977 Oct 15;109(2):317-29.

(225) Naito M, Hasegawa G, Ebe Y, Yamamoto T. Differentiation and function of Kupffer cells. Med Electron Microsc 2004 Mar;37(1):16-28.

(226) Wang YY, Myhre AE, Pettersen SJ, Dahle MK, Foster SJ, Thlemermann C, et al. Peptidoglycan of Staphyloccus aureus induces enhanced levels of matrix metalloproteinase-9 in human blood originating from neutrophils. Shock 2005 Sep;24(3):214-8.

(227) Phan HM, Gao MH, Lai NC, Tang T, Hammond HK. New signaling pathways associated with increased cardiac adenylyl cyclase 6 expression: implications for possible congestive heart failure therapy. Trends Cardiovasc Med 2007 Oct;17(7):215-21.

(228) Taussig R, Gilman AG. Mammalian membrane-bound adenylyl cyclases. J Biol Chem 1995 Jan 6;270(1):1-4.

(229) Wang YY, Dahle MK, Agren J, Myhre AE, Reinholt FP, Foster SJ, et al. Activation of the liver X receptor protects against hepatic injury in endotoxemia by suppressing Kupffer cell activation. Shock 2006 Feb;25(2):141-6.

(230) Risoe PK, Wang Y, Stuestol JF, Aasen AO, Wang JE, Dahle MK. Lipopolysaccharide attenuates mRNA levels of several adenylyl cyclase isoforms in vivo. Biochim Biophys Acta 2007 Jan;1772(1):32-9.

(231) Okonogi K, Gettys TW, Uhing RJ, Tarry WC, Adams DO, Prpic V. Inhibition of prostaglandin E2-stimulated cAMP accumulation by lipopolysaccharide in murine peritoneal macrophages. J Biol Chem 1991 Jun 5;266(16):10305-12.

(232) Nemeth K, Leelahavanichkul A, Yuen PS, Mayer B, Parmelee A, Doi K, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med 2009 Jan;15(1):42-9.

(233) Chung J, Serezani CH, Huang SK, Stern JN, Keskin DB, Jagirdar R, et al. Rap1 activation is required for Fc gamma receptor-dependent phagocytosis. J Immunol 2008 Oct 15;181(8):5501-9.

(234) Bystrom J, Evans I, Newson J, Stables M, Toor I, van RN, et al. Resolution-phase macrophages possess a unique inflammatory phenotype that is controlled by cAMP. Blood 2008 Nov 15;112(10):4117-27.

(235) Duan B, Davis R, Sadat EL, Collins J, Sternweis PC, Yuan D, et al. Distinct roles of adenylyl cyclase VII in regulating the immune responses in mice. J Immunol 2010 Jul 1;185(1):335-44.

(236) Risoe PK, Sitkovskiy A, Agren J, Ryg U, Valen G, Dahle MK. Gender differences in TNFa responses are associated with attenuated adenylyl cyclase expression in male monocytes. Submitted 2012.

(237) Risoe PK, Ryg U, Wang YY, Rutkovskiy A, Smedsrod B, Valen G, et al. Cecal ligation and puncture sepsis is associated with attenuated expression of adenylyl cyclase 9 and increased miR142-3p. Shock 2011 Oct;36(4):390-5.

(238) Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007 Jun;9(6):654-9.

(239) Akao Y, Iio A, Itoh T, Noguchi S, Itoh Y, Ohtsuki Y, et al. Microvesicle-mediated RNA molecule delivery system using monocytes/macrophages. Mol Ther 2011 Feb;19(2):395-9.

(240) Huang B, Zhao J, Lei Z, Shen S, Li D, Shen GX, et al. miR-142-3p restricts cAMP production in CD4+. EMBO Rep 2009 Feb;10(2):180-5.

(241) Merkerova M, Belickova M, Bruchova H. Differential expression of microRNAs in hematopoietic cell lineages. Eur J Haematol 2008 Oct;81(4):304-10.

(242) Laterza OF, Lim L, Garrett-Engele PW, Vlasakova K, Muniappa N, Tanaka WK, et al. Plasma MicroRNAs as sensitive and specific biomarkers of tissue injury. Clin Chem 2009 Nov;55(11):1977-83.

(243) Pauley KM, Stewart CM, Gauna AE, Dupre LC, Kuklani R, Chan AL, et al. Altered miR-146a expression in Sjogren's syndrome and its functional role in innate immunity. Eur J Immunol 2011 Jul;41(7):2029-39.

(244) Keller A, Leidinger P, Lange J, Borries A, Schroers H, Scheffler M, et al. Multiple sclerosis: microRNA expression profiles accurately differentiate patients with relapsing-remitting disease from healthy controls. PLoS ONE 2009;4(10):e7440.

(245) Wang XS, Gong JN, Yu J, Wang F, Zhang XH, Yin XL, et al. MicroRNA-29a and microRNA-142-3p are regulators of myeloid differentiation and acute myeloid leukemia. Blood 2012 May 24;119(21):4992-5004.

(246) Lv M, Zhang X, Jia H, Li D, Zhang B, Zhang H, et al. An oncogenic role of miR-142-3p in human T-cell acute lymphoblastic leukemia (T-ALL) by targeting glucocorticoid receptor-alpha and cAMP/PKA pathways. Leukemia 2012 Apr;26(4):769-77.

(247) Schaefer JS, Montufar-Solis D, Vigneswaran N, Klein JR. Selective upregulation of microRNA expression in peripheral blood leukocytes in IL-10-/- mice precedes expression in the colon. J Immunol 2011 Dec 1;187(11):5834-41.

(248) Danger R, Pallier A, Giral M, Martinez-Llordella M, Lozano JJ, Degauque N, et al. Upregulation of miR-142-3p in peripheral blood mononuclear cells of operationally tolerant patients with a renal transplant. J Am Soc Nephrol 2012 Apr;23(4):597-606.

(249) Sun Y, Varambally S, Maher CA, Cao Q, Chockley P, Toubai T, et al. Targeting of microRNA-142-3p in dendritic cells regulates endotoxin-induced mortality. Blood 2011 Jun 9;117(23):6172-83.

(250) Okumura S, Kawabe J, Yatani A, Takagi G, Lee MC, Hong C, et al. Type 5 adenylyl cyclase disruption alters not only sympathetic but also parasympathetic and calcium-mediated cardiac regulation. Circ Res 2003 Aug 22;93(4):364-71.

(251) Ishikawa Y, Katsushika S, Chen L, Halnon NJ, Kawabe J, Homcy CJ. Isolation and characterization of a novel cardiac adenylylcyclase cDNA. J Biol Chem 1992 Jul 5;267(19):13553-7.

(252) Katsushika S, Chen L, Kawabe J, Nilakantan R, Halnon NJ, Homcy CJ, et al. Cloning and characterization of a sixth adenylyl cyclase isoform: types V and VI constitute a subgroup within the mammalian adenylyl cyclase family. Proc Natl Acad Sci U S A 1992 Sep 15;89(18):8774-8.

(253) Tobise K, Ishikawa Y, Holmer SR, Im MJ, Newell JB, Yoshie H, et al. Changes in type VI adenylyl cyclase isoform expression correlate with a decreased capacity for cAMP generation in the aging ventricle. Circ Res 1994 Apr;74(4):596-603.

(254) Ping P, Anzai T, Gao M, Hammond HK. Adenylyl cyclase and G protein receptor kinase expression during development of heart failure. Am J Physiol 1997 Aug;273(2 Pt 2):H707-H717.

(255) Okumura S, Suzuki S, Ishikawa Y. New Aspects for the Treatment of Cardiac Diseases Based on the Diversity of Functional Controls on Cardiac Muscles: Effects of Targeted Disruption of the Type 5 Adenylyl Cyclase Gene. J Pharmacol Sci 2009 Mar 7.

(256) Phan HM, Gao MH, Lai NC, Tang T, Hammond HK. New signaling pathways associated with increased cardiac adenylyl cyclase 6 expression: implications for possible congestive heart failure therapy. Trends Cardiovasc Med 2007 Oct;17(7):215-21.

(257) Bristow MR, Ginsburg R, Minobe W, Cubicciotti RS, Sageman WS, Lurie K, et al. Decreased catecholamine sensitivity and beta-adrenergic-receptor density in failing human hearts. N Engl J Med 1982 Jul 22;307(4):205-11.

(258) Feldman MD, Copelas L, Gwathmey JK, Phillips P, Warren SE, Schoen FJ, et al. Deficient production of cyclic AMP: pharmacologic evidence of an important cause of contractile dysfunction in patients with end-stage heart failure. Circulation 1987 Feb;75(2):331-9.

(259) Espinasse I, Iourgenko V, Richer C, Heimburger M, Defer N, Bourin MC, et al. Decreased type VI adenylyl cyclase mRNA concentration and Mg(2+)-dependent adenylyl cyclase activities and unchanged type V adenylyl cyclase mRNA concentration and Mn(2+)-dependent adenylyl cyclase activities in the left ventricle of rats with myocardial infarction and longstanding heart failure. Cardiovasc Res 1999 Apr;42(1):87-98.

(260) Gao MH, Lai NC, Roth DM, Zhou J, Zhu J, Anzai T, et al. Adenylylcyclase increases responsiveness to catecholamine stimulation in transgenic mice. Circulation 1999 Mar 30;99(12):1618-22.

(261) Gao M, Ping P, Post S, Insel PA, Tang R, Hammond HK. Increased expression of adenylylcyclase type VI proportionately increases beta-adrenergic receptor-stimulated production of cAMP in neonatal rat cardiac myocytes. Proc Natl Acad Sci U S A 1998 Feb 3;95(3):1038-43.

(262) Gao MH, Tang T, Guo T, Sun SQ, Feramisco JR, Hammond HK. Adenylyl cyclase type VI gene transfer reduces phospholamban expression in cardiac myocytes via activating transcription factor 3. J Biol Chem 2004 Sep 10;279(37):38797-802.

(263) Roth DM, Bayat H, Drumm JD, Gao MH, Swaney JS, Ander A, et al. Adenylyl cyclase increases survival in cardiomyopathy. Circulation 2002 Apr 23;105(16):1989-94.

(264) Takahashi T, Tang T, Lai NC, Roth DM, Rebolledo B, Saito M, et al. Increased cardiac adenylyl cyclase expression is associated with increased survival after myocardial infarction. Circulation 2006 Aug 1;114(5):388-96.

(265) Roth DM, Gao MH, Lai NC, Drumm J, Dalton N, Zhou JY, et al. Cardiac-directed adenylyl cyclase expression improves heart function in murine cardiomyopathy. Circulation 1999 Jun 22;99(24):3099-102.

(266) Sastry A, Arnold E, Gurji H, Iwasa A, Bui H, Hassankhani A, et al. Cardiac-directed expression of adenylyl cyclase VI facilitates atrioventricular nodal conduction. J Am Coll Cardiol 2006 Aug 1;48(3):559-65.

(267) Lai NC, Tang T, Gao MH, Saito M, Takahashi T, Roth DM, et al. Activation of cardiac adenylyl cyclase expression increases function of the failing ischemic heart in mice. J Am Coll Cardiol 2008 Apr 15;51(15):1490-7.

(268) Liu X, Thangavel M, Sun SQ, Kaminsky J, Mahautmr P, Stitham J, et al. Adenylyl cyclase type 6 overexpression selectively enhances beta-adrenergic and prostacyclin receptor-mediated inhibition of cardiac fibroblast function because of colocalization in lipid rafts. Naunyn Schmiedebergs Arch Pharmacol 2008 Jun;377(4-6):359-69.

(269) Tang T, Gao MH, Roth DM, Guo T, Hammond HK. Adenylyl cyclase type VI corrects cardiac sarcoplasmic reticulum calcium uptake defects in cardiomyopathy. Am J Physiol Heart Circ Physiol 2004 Nov;287(5):H1906-H1912.

(270) Gao MH, Tang T, Guo T, Sun SQ, Feramisco JR, Hammond HK. Adenylyl cyclase type VI gene transfer reduces phospholamban expression in cardiac myocytes via activating transcription factor 3. J Biol Chem 2004 Sep 10;279(37):38797-802.

(271) Tang T, Gao MH, Lai NC, Firth AL, Takahashi T, Guo T, et al. Adenylyl cyclase type 6 deletion decreases left ventricular function via impaired calcium handling. Circulation 2008 Jan 1;117(1):61-9.

(272) Bogatcheva NV, Garcia JG, Verin AD. Molecular mechanisms of thrombin-induced endothelial cell permeability. Biochemistry (Mosc) 2002 Jan;67(1):75-84.

(273) Patterson CE, Lum H, Schaphorst KL, Verin AD, Garcia JG. Regulation of endothelial barrier function by the cAMP-dependent protein kinase. Endothelium 2000;7(4):287-308.

(274) Cioffi DL, Moore TM, Schaack J, Creighton JR, Cooper DM, Stevens T. Dominant regulation of interendothelial cell gap formation by calcium-inhibited type 6 adenylyl cyclase. J Cell Biol 2002 Jun 24;157(7):1267-78.

(275) Birukova AA, Zagranichnaya T, Fu P, Alekseeva E, Chen W, Jacobson JR, et al. Prostaglandins PGE(2) and PGI(2) promote endothelial barrier enhancement via PKA- and Epac1/Rap1-dependent Rac activation. Exp Cell Res 2007 Jul 1;313(11):2504-20.

(276) Hordijk PL. Endothelial signalling events during leukocyte transmigration. FEBS J 2006 Oct;273(19):4408-15.

(277) Manolopoulos VG, Fenton JW, Lelkes PI. The thrombin receptor in adrenal medullary microvascular endothelial cells is negatively coupled to adenylyl cyclase through a Gi protein. Biochim Biophys Acta 1997 May 27;1356(3):321-32.

(278) Geraud C, Evdokimov K, Straub BK, Peitsch WK, Demory A, Dorflinger Y, et al. Unique cell type-specific junctional complexes in vascular endothelium of human and rat liver sinusoids. PLoS One 2012;7(4):e34206.

(279) Geraud C, Schledzewski K, Demory A, Klein D, Kaus M, Peyre F, et al. Liver sinusoidal endothelium: a microenvironment-dependent differentiation program in rat including the novel junctional protein liver endothelial differentiation-associated protein-1. Hepatology 2010 Jul;52(1):313-26.

(280) Saito M, Matsuura T, Nagatsuma K, Tanaka K, Maehashi H, Shimizu K, et al. The functional interrelationship between gap junctions and fenestrae in endothelial cells of the liver organoid. J Membr Biol 2007 Jun;217(1-3):115-21.

(281) Semaeva E, Tenstad O, Skavland J, Enger M, Iversen PO, Gjertsen BT, et al. Access to the spleen microenvironment through lymph shows local cytokine production, increased cell flux, and altered signaling of immune cells during lipopolysaccharide-induced acute inflammation. J Immunol 2010 Apr 15;184(8):4547-56.

(282) Robinette CD, Fraumeni JF, Jr. Splenectomy and subsequent mortality in veterans of the 1939-45 war. Lancet 1977 Jul 16;2(8029):127-9.

(283) Huang B, Zhao J, Lei Z, Shen S, Li D, Shen GX, et al. miR-142-3p restricts cAMP production in CD4+. EMBO Rep 2009 Feb;10(2):180-5.

(284) Szabo G, Romics L, Jr., Frendl G. Liver in sepsis and systemic inflammatory response syndrome. Clin Liver Dis 2002 Nov;6(4):1045-66, x.

(285) Lee WY, Kubes P. Leukocyte adhesion in the liver: distinct adhesion paradigm from other organs. J Hepatol 2008 Mar;48(3):504-12.

(286) Wisse E, De Zanger RB, Charels K, Van Der SP, McCuskey RS. The liver sieve: considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Disse. Hepatology 1985 Jul;5(4):683-92.

(287) Wisse E, Luo D, Vermijlen D, Kanellopoulou C, De ZR, Braet F. On the function of pit cells, the liver-specific natural killer cells. Semin Liver Dis 1997;17(4):265-86.

(288) Minshall RD, Malik AB. Transport across the endothelium: regulation of endothelial permeability. Handb Exp Pharmacol 2006;(176 Pt 1):107-44.

(289) Cioffi DL, Moore TM, Schaack J, Creighton JR, Cooper DMF, Stevens T. Dominant regulation of interendothelial cell gap formation by calcium-inhibited type 6 adenylyl cyclase. Journal of Cell Biology 2002 Jun 24;157(7):1267-78.

(290) Aulock SV, Deininger S, Draing C, Gueinzius K, Dehus O, Hermann C. Gender difference in cytokine secretion on immune stimulation with LPS and LTA. J Interferon Cytokine Res 2006 Dec;26(12):887-92.

(291) van Eijk LT, Dorresteijn MJ, Pikkers P. Gender influences in vivo human responses to endotoxin. Shock 2008 Mar;29(3):422-3.

(292) Goetzl EJ, Huang MC, Kon J, Patel K, Schwartz JB, Fast K, et al. Gender specificity of altered human immune cytokine profiles in aging. FASEB J 2010 Sep;24(9):3580-9.

(293) Bouman A, Heineman MJ, Faas MM. Sex hormones and the immune response in humans. Hum Reprod Update 2005 Jul;11(4):411-23.

(294) Moxley G, Posthuma D, Carlson P, Estrada E, Han J, Benson LL, et al. Sexual dimorphism in innate immunity. Arthritis Rheum 2002 Jan;46(1):250-8.

(295) Ralston SH, Russell RG, Gowen M. Estrogen inhibits release of tumor necrosis factor from peripheral blood mononuclear cells in postmenopausal women. J Bone Miner Res 1990 Sep;5(9):983-8.

(296) Shanker G, Sorci-Thomas M, Adams MR. Estrogen modulates the inducible expression of platelet-derived growth factor mRNA by monocyte/macrophages. Life Sci 1995;56(7):499-507.

(297) Puder JJ, Freda PU, Goland RS, Wardlaw SL. Estrogen modulates the hypothalamic-pituitary-adrenal and inflammatory cytokine responses to endotoxin in women. J Clin Endocrinol Metab 2001 Jun;86(6):2403-8.

(298) Kawasaki T, Chaudry IH. The effects of estrogen on various organs: therapeutic approach for sepsis, trauma, and reperfusion injury. Part 1: central nervous system, lung, and heart. J Anesth 2012 Jun 23.

(299) Sekido R, Murai K, Funahashi J, Kamachi Y, Fujisawa-Sehara A, Nabeshima Y, et al. The delta-crystallin enhancer-binding protein delta EF1 is a repressor of E2-box-mediated gene activation. Mol Cell Biol 1994 Sep;14(9):5692-700.

(300) Dillner NB, Sanders MM. Transcriptional activation by the zinc-finger homeodomain protein delta EF1 in estrogen signaling cascades. DNA Cell Biol 2004 Jan;23(1):25-34.

(301) Duan B, Davis R, Sadat EL, Collins J, Sternweis PC, Yuan D, et al. Distinct roles of adenylyl cyclase VII in regulating the immune responses in mice. J Immunol 2010 Jul 1;185(1):335-44.

(302) Dahle MK, Myhre AE, Aasen AO, Wang JE. Effects of forskolin on Kupffer cell production of interleukin-10 and tumor necrosis factor alpha differ from those of endogenous adenylyl cyclase activators: possible role for adenylyl cyclase 9. Infect Immun 2005 Nov;73(11):7290-6.

(303) Bryn T, Yaqub S, Mahic M, Henjum K, Aandahl EM, Tasken K. LPS-activated monocytes suppress T-cell immune responses and induce FOXP3+ T cells through a COX-2-PGE2-dependent mechanism. Int Immunol 2008 Feb;20(2):235-45.

(304) Martinet L, Jean C, Dietrich G, Fournie JJ, Poupot R. PGE2 inhibits natural killer and gamma delta T cell cytotoxicity triggered by NKR and TCR through a cAMP-mediated PKA type I-dependent signaling. Biochem Pharmacol 2010 Sep 15;80(6):838-45.

(305) Lorenowicz MJ, Fernandez-Borja M, Hordijk PL. cAMP signaling in leukocyte transendothelial migration. Arterioscler Thromb Vasc Biol 2007 May;27(5):1014-22.

(306) Richter J. Effect of adenosine analogues and cAMP-raising agents on TNF-, GM-CSF-, and chemotactic peptide-induced degranulation in single adherent neutrophils. J Leukoc Biol 1992 Mar;51(3):270-5.

(307) Thivierge M, Alami N, Muller E, de Brum-Fernandes AJ, Rola-Pleszczynski M. Transcriptional modulation of platelet-activating factor receptor gene expression by cyclic AMP. J Biol Chem 1993 Aug 15;268(23):17457-62.

(308) Cioffi DL, Moore TM, Schaack J, Creighton JR, Cooper DM, Stevens T. Dominant regulation of interendothelial cell gap formation by calcium-inhibited type 6 adenylyl cyclase. J Cell Biol 2002 Jun 24;157(7):1267-78.

(309) Pavan B, Biondi C, Dalpiaz A. Adenylyl cyclases as innovative therapeutic goals. Drug Discov Today 2009 Oct;14(19-20):982-91.

(310) Pierre S, Eschenhagen T, Geisslinger G, Scholich K. Capturing adenylyl cyclases as potential drug targets. Nat Rev Drug Discov 2009 Apr;8(4):321-35.

(311) Defer N, Best-Belpomme M, Hanoune J. Tissue specificity and physiological relevance of various isoforms of adenylyl cyclase. American Journal of Physiology-Renal Physiology 2000 Sep;279(3):F400-F416.

(312) Horrigan LA, Kelly JP, Connor TJ. Immunomodulatory effects of caffeine: friend or foe? Pharmacol Ther 2006 Sep;111(3):877-92.

(313) Coimbra R, Melbostad H, Loomis W, Tobar M, Hoyt DB. Phosphodiesterase inhibition decreases nuclear factor-kappaB activation and shifts the cytokine response toward anti-inflammatory activity in acute endotoxemia. J Trauma 2005 Sep;59(3):575-82.

(314) Jilg S, Barsig J, Leist M, Kusters S, Volk HD, Wendel A. Enhanced release of interleukin-10 and soluble tumor necrosis factor receptors as novel principles of methylxanthine action in murine models of endotoxic shock. J Pharmacol Exp Ther 1996 Jul;278(1):421-31.

(315) Currie MS, Rao KM, Padmanabhan J, Jones A, Crawford J, Cohen HJ. Stimulus-specific effects of pentoxifylline on neutrophil CR3 expression, degranulation, and superoxide production. J Leukoc Biol 1990 Mar;47(3):244-50.

(316) Barroso-Aranda J, Schmid-Schonbein GW. Pentoxifylline pretreatment decreases the pool of circulating activated neutrophils, in-vivo adhesion to endothelium, and improves survival from hemorrhagic shock. Biorheology 1990;27(3-4):401-18.

(317) Hammerschmidt DE, Kotasek D, McCarthy T, Huh PW, Freyburger G, Vercellotti GM. Pentoxifylline inhibits granulocyte and platelet function, including granulocyte priming by platelet activating factor. J Lab Clin Med 1988 Aug;112(2):254-63.

(318) Bahra PS, Rainger GE, Wautier JL, Nash GB. Effects of pentoxifylline on the different steps during adhesion and transendothelial migration of flowing neutrophils. Cell Biochem Funct 2001 Dec;19(4):249-57.

(319) Michetti C, Coimbra R, Hoyt DB, Loomis W, Junger W, Wolf P. Pentoxifylline reduces acute lung injury in chronic endotoxemia. J Surg Res 2003 Nov;115(1):92-9.

(320) Coimbra R, Loomis W, Melbostad H, Tobar M, Porcides RD, Hoyt DB. LPS-stimulated PMN activation and proinflammatory mediator synthesis is downregulated by phosphodiesterase inhibition: role of pentoxifylline. J Trauma 2004 Dec;57(6):1157-63.

(321) Castro A, Jerez MJ, Gil C, Martinez A. Cyclic nucleotide phosphodiesterases and their role in immunomodulatory responses: advances in the development of specific phosphodiesterase inhibitors. Med Res Rev 2005 Mar;25(2):229-44.

(322) Jin SL, Lan L, Zoudilova M, Conti M. Specific role of phosphodiesterase 4B in lipopolysaccharide-induced signaling in mouse macrophages. J Immunol 2005 Aug 1;175(3):1523-31.

(323) Smith PG, Wang F, Wilkinson KN, Savage KJ, Klein U, Neuberg DS, et al. The phosphodiesterase PDE4B limits cAMP-associated PI3K/AKT-dependent apoptosis in diffuse large B-cell lymphoma. Blood 2005 Jan 1;105(1):308-16.

(324) Dastidar SG, Rajagopal D, Ray A. Therapeutic benefit of PDE4 inhibitors in inflammatory diseases. Curr Opin Investig Drugs 2007 May;8(5):364-72.

(325) Spina D. PDE4 inhibitors: current status. Br J Pharmacol 2008 Oct;155(3):308-15.

(326) Schoof CR, Botelho EL, Izzotti A, Vasques LR. MicroRNAs in cancer treatment and prognosis. Am J Cancer Res 2012;2(4):414-33.

(327) Lanford RE, Hildebrandt-Eriksen ES, Petri A, Persson R, Lindow M, Munk ME, et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science 2010 Jan 8;327(5962):198-201.

(328) Redis RS, Calin S, Yang Y, You MJ, Calin GA. Cell-to-cell miRNA transfer: From body homeostasis to therapy. Pharmacol Ther 2012 Aug 8.

(329) Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, et al. Silencing of microRNAs in vivo with 'antagomirs'. Nature 2005 Dec 1;438(7068):685-9.

(330) Vester B, Wengel J. LNA (locked nucleic acid): high-affinity targeting of complementary RNA and DNA. Biochemistry 2004 Oct 26;43(42):13233-41.

(331) Ebert MS, Neilson JR, Sharp PA. MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. Nat Methods 2007 Sep;4(9):721-6.

(332) Weiler J, Hunziker J, Hall J. Anti-miRNA oligonucleotides (AMOs): ammunition to target miRNAs implicated in human disease? Gene Ther 2006 Mar;13(6):496-502.

(333) Miyazaki Y, Adachi H, Katsuno M, Minamiyama M, Jiang YM, Huang Z, et al. Viral delivery of miR-196a ameliorates the SBMA phenotype via the silencing of CELF2. Nat Med 2012 Jun 3.

7. Paper I

Risøe PK, Wang YY, Stuestøl JF, Aasen AO, Wang JE, Dahle MK. *Lipopolysaccharide attenuates mRNA levels of several adenylyl cyclase isoforms in vivo.* Biochem. Biophys. Acta 2007 Jan;1772(1):32-9.

8. Paper II

Risøe PK, Ryg U, Wang YY, Rutkovskiy A, Smedsrød B, Valen G, Dahle MK. *Cecal ligation and puncture sepsis is associated with attenuated expression of adenylyl cyclase 9 and increased miR142-3p.* Shock 2011 Oct;36(4):390-5.

9. Paper III

Risøe PK, Rutkovskiy A, Ågren J, Kolseth IB, Flood Kjeldsen S, Valen G, Vaage J, Dahle MK. *Gender differences in TNFα responses are associated with attenuated adenylyl cyclase expression in male monocytes.* Manuscript submitted for publication.